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ANDREW STUART MITCHELL, 1864-1932

ANDREW STUART MITCHELL

On a rainy day in August a silent and sorrowful group of people gathered at the Virginia home of Andrew S. Mitchell to pay a last tribute to one who as coworker, advisor, or friend had won the esteem, confidence, and affection of everyone present.

Mr. Mitchell died on August 17, 1932, after a long illness of a kind that saps the vitals and makes cowards of the bravest, but which he faced with a cheerful courage that amazed even those who best knew his sturdy nature and powers of endurance. Many months before the end came, medical experts had pronounced sentence of death, but this knowledge was buried deep within his own soul and as long as consciousness remained and speech was possible, he was the same humorous, whimsical, lovable "Andy" Mitchell his intimates knew in health, ready with jest or story or sympathy for another's trouble. No waving banners or martial strains urged on this valiant warrior in the battle he knew to be a losing one, but his indomitable spirit never faltered and he went down to defeat with colors flying.

At the time he was forced by illness to retire, Mr. Mitchell was an advisory chemist in the Food and Drug Administration, Department of Agriculture. He entered the Federal service in 1907, as Chief of the St. Paul Food and Drug Inspection Laboratory. Special technical courses in the University of Michigan and eight years of service as State Chemist of Wisconsin peculiarly fitted him for duty in connection with the enforcement of a measure regulating commerce in foods and drugs.

After five years in St. Paul he was transferred to Washington to act as a member of the Board of Food and Drug Inspection, created by the Secretary of Agriculture to consider all questions arising in the enforcement of the food and drugs act, and later he was named a member of the committee appointed to prepare regulations for the net weight amendment to that act. At every stage of his quarter century of service with the Government Mr. Mitchell was intimately associated with the projects and policies of the different organizations with which he was connected. As a member of the Food Standards Committee, appointed by the Secretary of Agriculture to define and propose standards for foods, his work was also outstanding and far-reaching. He was for eleven years its secretary.

In the chaotic days of the Great War his impartial judgment and technical knowledge were frequently called into play to cooperate with various war-time agencies. A broad knowledge of grains and grain products, acquired through long years spent in the heart of the American milling industry, qualified him to represent the Government in defending a charge brought by a South American firm that a shipment of flour delivered through the United States Shipping Board was damaged. The War Trade Board also availed itself of his knowledge of chemicals in considering applications for license to import or export chemicals, the question at issue being whether such chemicals could be used as munitions of war. He acted as advisor to the Board until the signing of the Armistice made such service unnecessary.

Foods and drugs were not the only subjects of his many investigations and studies. During the years he was State Chemist of Wisconsin he maintained a separate laboratory where, as a consulting chemist, he

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carried on work in widely diversified lines. He early recognized the value of the Roentgen ray in the field of therapeutics and owned one of the first pieces of x-ray apparatus operated in the West. It was in this laboratory that he spent many of the late hours of the night in gratifying his love of music and in indulging a taste acquired in his boyhood for certain hobbies. Nothing more eloquently bespeaks the character of the man than do these simple tastes.

Born in Milwaukee in 1864, Andrew Stuart Mitchell spent all of his boyhood there and many of the years of his early manhood. Those were happy days. He played the flute, sang bass in a local church choir, and catered to his scientific bent by much rambling around in search of botanical specimens. These homely pleasures were characteristic of those of later years. A rare stamp was to him of greater value than a precious gem, and he treasured an Indian relic as a miser does his gold. Quite early he commenced to collect stamps, and his collection is pronounced by experts to be a valuable one. The Indian relics accumulated over a period of many years were secured by him only after long tramps to the mounds in his own and neighboring States. The collection is a fine one and is to be presented to the Wisconsin State Historical Society. The grounds of his home on the Potomac are mute but indisputable witnesses of his absorbing love of trees and flowers. Any bird could be sure of protection in his trees, and a stray dog had only to be hungry and homeless to get a passport to food and affection if he chose to stick around. In sharp contrast to his craving for the beautiful and his boundless charity for the unfortunate were his unwavering adherence to a principle, his insistence upon accuracy of detail, his unyielding attitude when he had once decided that a given course was right.

Mr. Mitchell was twice married. His first wife was Margaret Cheyne, of Milwaukee. She died during the flu epidemic following the war. Two children were born to them, Norman Stuart and Margaret Marcella. Both are living. In 1920 he married Florence Quincy Norton, of Madison, Wis., who survives him.

Much could be written of the scientific attainments and personal characteristics of Andy Mitchell, but the real man was known only to those who had the privilege of seeing him in his own home, a roomy, rambling house standing high on a knoll overlooking the Potomac. Under the mellowing influence of a harmonious domestic atmosphere his genial attributes held full sway. The Mitchell home was a rallying place for the many friends who enjoyed its charming hospitality.

Andy Mitchell is gone, but the memory of a pronounced personality will remain long with those who knew him. Steadfast in his devotion to duty as he saw it, firm to the point of stubbornness in his convictions, loyal to principles, he was nevertheless an entertaining companion, a perfect host, a friend under any kind of sky, an ideal husband and father, in short, a man!

G. L. BIDWELL

PROCEEDINGS OF THE FORTY-EIGHTH ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1932

The forty-eighth annual convention of the Association of Official Agricultural Chemists was held at the Raleigh Hotel, Washington D.C., October 31, November 1 and 2, 1932.

The meeting was called to order by the president, A. E. Paul, U. S. Food and Drug Administration, Chicago, Illinois, on the morning of October 31, at 10:30 o'clock.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR ENDING OCTOBER, 1933

President

J. W. Kellogg, Department of Agriculture, Harrisburg, Pa.

Vice-President

R. Harcourt, Guelph, Canada

Secretary-Treasurer

W. W. Skinner, Bureau of Chemistry and Soils, Washington, D. C.

Additional Members of the Executive Committee

F. C. Blanck, Washington, D. C.
H. H. Hanson, Dover, Del.
C. C. McDonnell, Washington, D. C.
A. E. Paul, Chicago, Ill.

Permanent Committees
Recommendations of Referees

(Figures in parentheses refer to year in which appointment expires.)

E. M. Bailey (Agricultural Experiment Station, New Haven, Conn.), *Chairman*

Subcommittee A: H. H. Hanson (1934), (State Board of Agriculture, Dover, Del.), *Chairman;* H. R. Kraybill (1936); G. L. Bidwell (1938). [Insecticides, fungicides, and caustic poisons (flourine compounds); soils and liming materials (hydrogen-ion concentration, alkaline soils and acid soils; liming materials, less common metals in soils); feeding stuffs (stock feed adulteration, mineral mixed feeds, moisture, hydrocyanic acid in glucoside-bearing materials, solvents for determination of fat; biological methods for determination of cod-liver oil in feed mixtures, fat in dairy products used as feeds); sugars and sugar

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products (honey, maple products; drying, densimetric, and refractometric methods; polariscopic methods; chemical methods for reducing sugars); fertilizers (phosphoric acid, nitrogen, high analysis fertilizers, potash); plants (less common metals, total chlorine, carbohydrates, forms of nitrogen, sodium); lignin, enzymes, paints.]

SUBCOMMITTEE B: A. G. Murray (1934), (U.S. Food and Drug Administration, Washington, D. C.), *Chairman;* L. E. Warren (1936); L. B. Broughton (1938). [Naval stores (resin, turpentine); drugs (crude drugs, radioactivity in foods and drugs, mercurials, microchemical methods for alkaloids, hypophosphites, santonin, ether, benzyl compounds, small quantities of morphine in sirups, guaiacol, bromide-bromate methods; rhubarb and rhaponticum, calcium gluconate, tetrachlorethylene, hexylresorcinol, ergot alkaloids, microchemical methods for synthetics, nitrites in tablets, ointments, biological testing, acetphenetidin in presence of caffeine and aspirin, strychnine in tablets, resins and oleoresins, pyridium, gums, essential oils); beers, wines, and distilled liquors.]

SUBCOMMITTEE C: G. G. Frary (1934), (Department of Agriculture, Vermilion, S. D.), *Chairman;* H. A. Lepper (1936); J. O. Clarke (1938). [Dairy products (milk, butter, cheese, malted milk, dried milk, ice cream, milk proteins); fats and oils (oil in oleaginous seeds); eggs and egg products (reducing sugars, sucrose, added salt, fat, lipoids, P_2O_5 and crude albumin nitrogen; detection of decomposition, glycerol, unsaponifiable matter); food preservatives, (sulfurous acid in dried fruits), coloring matters in foods, metals in foods (arsenic, bromate method; arsenic, arsine distillation and Gutzeit methods; copper and zinc, fluorine, lead), fruits and fruit products (soluble solids, ash, fruit acids, effect of H-ion concentration on extraction of fruits, moisture in dried fruits), canned foods, vinegars, flavors and non-alcoholic beverages, meats and meat products (separation of meat proteins), gelatin, cacao products (milk proteins in milk chocolate, cacao butter, sucrose and lactose in milk chocolate), coffee, gums in foods, spices and other condiments (volatile oil in spices), baking powders and baking chemicals, cereal foods (ash in flour, alimentary paste and baked products; chloride in flour and baked products, and color in flour; H-ion concentration of flour, diastatic value of flour, starch in flour, flour-bleaching chemicals, foreign methods for testing flour, CO_2 in self-rising flour, sampling alimentary pastes and determination of moisture in baked products, crude fiber in baked products, methods for flour, alimentary pastes and baked products—(a) unsaponifiable matter, (b) fat, crude albumin nitrogen, lipoids, P_2O_5; milk solids in milk bread, rye in flour mixtures, experimental baking tests), microchemical methods, microbiological methods.]

Methods of Analysis

W. W. SKINNER, *Chairman*

J. A. LeCLERC   L. E. WARREN

J. W. SALE   MARIAN E. LAPP

G. G. FRARY

Principles and Practice of Agricultural Analysis

C. A. BROWNE and W. W. SKINNER (Bureau of Chemistry and
Soils, Washington, D. C.)

SPECIAL COMMITTEES

*Committee on Definition of Terms and Interpretation of Results on Fertilizers
and Liming Materials*

H. D. HASKINS (Agricultural Experiment Station, Amherst, Mass.), *Chairman*

C. H. JONES   G. S. FRAPS

J. W. KELLOGG   R. N. BRACKETT

W. H. MacINTIRE

Committee on Quartz-Plate Standardization and Normal Weight

FREDERICK BATES (Bureau of Standards, Washington, D. C.), *Chairman*

C. A. BROWNE   F. W. ZERBAN

Committee on Standard Scale for Immersion Refractometer

R. T. BALCH (Bureau of Chemistry and Soils, Washington, D. C.), *Chairman*

C. F. SNYDER   H. C. GORE

*Committee to Confer with American Public Health Association on Standard Methods
of Milk Analysis*

E. M. BAILEY (Agricultural Experiment Station, New Haven, Conn.), *Chairman*

G. G. FRARY   F. C. BLANCK

*Representatives on the Board of Governors of the Crop Protection Institute of
the National Research Council*

H. J. PATTERSON, College Park, Md.

W. H. MacINTIRE, Knoxville, Tenn.

Committee on Sampling

F. C. BLANCK (Bureau of Chemistry and Soils, Washington, D. C.), *Chairman;*
F. C. BLANCK, *Foods;* J. W. KELLOGG, *Feeding Stuffs;* A. E. PAUL, *Drugs;*
A. G. McCALL, *Soils and Liming Materials;* C. C. McDONNELL, *Insecticides
and Fungicides;* R. N. BRACKETT, *Fertilizers;* F. W. ZERBAN, *Saccharine
Products;* R. W. FREY, *Tanning Materials and Leathers;* J. W. SALE, *Water;*
O. B. WINTER, *Plants.*

REFEREES AND ASSOCIATE REFEREES

INSECTICIDES, FUNGICIDES, AND CAUSTIC POISONS

General referee: J. J. T. Graham, Food and Drug Adm., Washington, D. C.

FLUORINE COMPOUNDS:

Associate referee: G. A. Shuey, Agricultural Experiment Station, Knox-
ville, Tenn.

SOILS AND LIMING MATERIALS:

General referee: W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

HYDROGEN-ION CONCENTRATION

a. ALKALINE SOILS:

Associate referee: P. L. Hibbard, University of California, Berkeley, Calif.

b. ACID SOILS:

Associate referee: M. F. Morgan, Agricultural Experiment Station, New Haven, Conn.

LIMING MATERIALS:

Associate referee: W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.

LESS COMMON METALS IN SOILS:

Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

FEEDING STUFFS:

General referee: V. E. Munsey, Food and Drug Adm., Washington, D. C.

STOCK FEED ADULTERATION:

Associate referee: H. E. Gensler, Department of Agriculture, Harrisburg, Pa.

MINERAL MIXED FEEDS:

Associate referee: H. A. Halvorson, Old Capitol Building, St. Paul, Minn.

MOISTURE:

Associate referee: G. E. Grattan, Department of Agriculture, Ottawa, Canada

HYDROCYANIC ACID IN GLUCOSIDE-BEARING MATERIALS:

Associate referee: G. C. Smith, Food and Drug Adm., Washington, D. C.

SOLVENTS FOR DETERMINATION OF FAT IN FEEDING STUFFS:

Associate referee: L. S. Walker, Agricultural Experiment Station, Burlington, Vt.

BIOLOGICAL METHODS FOR THE DETERMINATION OF COD LIVER OIL IN FEED MIXTURES:

Associate referee: W. B. Griem, Department of Agriculture and Markets, Madison, Wis.

FAT IN DAIRY PRODUCTS USED AS FEEDS:

Associate referee: A. B. Heagy, College Park, Md.

SUGARS AND SUGAR PRODUCTS:

General referee: J. A. Ambler, Bureau of Chemistry and Soils, Washington, D. C.

HONEY:
> *Associate referee:* H. A. Schuette, University of Wisconsin, Madison, Wis.

MAPLE PRODUCTS:
> *Associate referee:* J. F. Snell, Macdonald College, Quebec, Canada.

DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS:
> *Associate referee:* C. F. Snyder, Bureau of Standards, Washington, D. C.

POLARISCOPIC METHODS:
> *Associate referee:* S. Byall, Bureau of Chemistry and Soils, Washington, D. C.

CHEMICAL METHODS FOR REDUCING SUGARS:
> *Associate referee:* R. F. Jackson, Bureau of Standards, Washington, D. C.

FERTILIZERS:
> *General referee:* G. S. Fraps, Agricultural Experiment Station, College Station, Tex.

PHOSPHORIC ACID:
> *Associate referee:* W. H. Ross, Bureau of Chemistry and Soils, Washington, D. C.

NITROGEN:
> *Associate referee:* A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

HIGH ANALYSIS FERTILIZERS:
> *Associate referee:* J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

POTASH
> *Associate referee:* L. D. Haigh, Agricultural Experiment Station, Columbia, Mo.

PLANTS:
> *General referee and associate referee on fluorine:* O. B. Winter, Agricultural Experiment Station, E. Lansing, Mich.

LESS COMMON METALS:
> *Associate referee:* J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

TOTAL CHLORINE:
> *Associate referee:* H. L. Wilkins, Bureau of Plant Industry, Washington, D. C.

CARBOHYDRATES:
> *Associate referee:* J. T. Sullivan, Agricultural Experiment Station, Purdue, Ind.

FORMS OF NITROGEN:
> *Associate referee:* H. B. Vickery, Agricultural Experiment Station, New Haven, Conn.

SODIUM:
> *Associate referee:* Lillian Butler, Agricultural Experiment Station, E. Lansing, Mich.

LIGNIN:
> *General referee:* M. Philips, Bureau of Chemistry and Soils, Washington, D. C.

ENZYMES:
> *General referee:* E. K. Balls, Bureau of Chemistry and Soils, Washington, D. C.

PAINTS, PAINT MATERIALS AND VARNISHES:
> *General referee:* C. S. Ladd, Office of Food Commissioner and Chemist, Bismarck, N. D.

NAVAL STORES:
> *General referee and associate referee on rosin:* F. P. Veitch, Bureau of Chemistry and Soils, Washington, D. C.

TURPENTINE:
> *Associate referee:* V. E. Grotlisch, Food and Drug Adm., Washington, D. C.

DRUGS:
> *General referee:* A. E. Paul, 1625 Transportation Bldg., Chicago, Ill.

CRUDE DRUGS:
> *Associate referee:* H. W. Youngken, Massachusetts College of Pharmacy, Boston, Mass.

RADIOACTIVITY IN FOODS AND DRUGS:
> *Associate referee:* C. H. Badger, Food and Drug Adm., Washington, D. C.

CALCIUM GLUCONATE:
> *Associate referee:* H. J. Fisher, Agricultural Experiment Station, New Haven, Conn.

MERCURIALS:
> *Associate referee:* E. C. Deal, Food and Drug Adm., New Orleans, La.

MICROCHEMICAL METHODS FOR ALKALOIDS:
> *Associate referee:* C. K. Glycart, Food and Drug Adm., Chicago, Ill.

MICROCHEMICAL METHODS FOR SYNTHETICS:
> *Associate referee:* I. S. Shupe, Food and Drug Adm., Chicago, Ill.

HYPOPHOSPHITES:
> *Associate referee:* H. R. Bond, Food and Drug Adm., Chicago, Ill.

SANTONIN:
> *Associate referee:* H. M. Burlage, School of Pharmacy, University of North Carolina, Chapel Hill, N. C.

ETHER:
> *Associate referee:* W. F. Kunke, Food and Drug Adm., Chicago, Ill.

BENZYL COMPOUNDS:
    Associate referee: J. Callaway, Jr., Food and Drug Adm., New York City

TETRACHLORETHYLENE:
    Associate referee: G. M. Johnson, Food and Drug Adm., Chicago, Ill.

SMALL QUANTITIES OF MORPHINE IN SIRUPS:
    Associate referee: E. O. Eaton, Food and Drug Adm., San Francisco, Calif.

HEXYLRESORCINOL:
    Associate referee: J. Carol, Food and Drug Adm., Chicago, Ill

ERGOT ALKALOIDS
    Associate referee: C. K. Glycart, Food and Drug Adm., Chicago, Ill.

GUAIACOL:
    Associate referee: N. L. Knight, Room 204, Old Custom House, St. Louis, Mo.

RHUBARB AND RHAPONTICUM:
    Associate referee: A. Viehoever, Philadelphia College of Pharmacy, Philadelphia, Pa.

BIOLOGICAL TESTING:
    Associate referee: W. T. McClosky, Food and Drug Adm., Washington, D. C.

BROMIDE-BROMATE METHODS:
    Associate referee: H. Wales, Food and Drug Adm., Washington, D. C.

NITRITES IN TABLETS:
    Associate referee: F. C. Sinton, Food and Drug Adm., New York City

OINTMENTS:
    Associate referee: W. F. Reindollar, State Dept. of Health, Baltimore, Md.

ACETPHENETIDIN IN PRESENCE OF CAFFEINE AND ASPIRIN:
    Associate referee: L. E. Warren, Food and Drug Adm., Washington, D. C.

STRYCHNINE IN TABLETS:
    Associate referee: C. W. Harrison, Food and Drug Adm., Minneapolis, Minn.

RESINS AND OLEORESINS:
    Associate referee: L. E. Warren.

PYRIDIUM:
    Associate referee: C. L. Clay, Dept. of Health, New Orleans, La.

GUMS:
    Associate referee: J. H. Cannon, Food and Drug Adm., Chicago, Ill.

ESSENTIAL OILS:
> *Associate referee:* Earl A. Anderson, Food and Drug Adm., New York City.

DAIRY PRODUCTS:
> *General referee and associate referee on milk:* G. G. Frary, Dairy and Food Dept., Vermilion, S. D.

BUTTER:
> *Associate referee:* C. W. Harrison, Food and Drug Adm., Minneapolis, Minn.

CHEESE:
> *Associate referee:* C. B. Stone, Food and Drug Adm., Minneapolis, Minn.

MALTED MILK:
> *Associate referee:* F. Hillig, Food and Drug Adm., Washington, D. C.

DRIED MILK
> *Associate referee:* Leslie Hart, Food and Drug Adm., Washington, D. C.

ICE CREAM:
> *Associate referee:* G. G. Frary.

MILK PROTEINS:
> *Associate referee:* M. L. Offutt, Food and Drug Adm., New York City.

FATS AND OILS:
> *General referee:* G. S. Jamieson, Bureau of Chemistry and Soils, Washington, D. C.

OIL IN OLEAGINOUS SEEDS:
> *Associate referee:* T. H. Hopper, Agricultural Experiment Station, Fargo, N. Dak.

EGGS AND EGG PRODUCTS:
> *General referee:* S. Alfend, Food and Drug Adm., St. Louis, Mo.

REDUCING SUGARS, SUCROSE, ADDED SALT, FAT, LIPOIDS, P_2O_5, AND CRUDE ALBUMIN NITROGEN
> *Associate referee:* L. C. Mitchell, Food and Drug Adm., St. Louis, Mo.

GLYCEROL AND UNSAPONIFIABLE MATTER:
> *Associate referee:* S. Alfend.

DETECTION OF DECOMPOSITION:
> *Associate referee:* H. D. Grigsby, Food and Drug Adm., New York City.

FOOD PRESERVATIVES:
> *General referee:* J. C. Krantz, Jr., State Department of Health, Baltimore, Md.

SULFUROUS ACID IN DRIED FRUITS:
> *Associate referee:* Paul Clifford, Food and Drug Adm., Washington, D. C.

COLORING MATTERS IN FOODS:
General referee: C. F. Jablonski, Food and Drug Adm., New York City.

METALS IN FOODS:
General referee: H. J. Wichmann, Food and Drug Adm., Washington, D. C.

 ARSENIC
  a. BROMATE METHOD:
   Associate referee: W. C. Jones, Department of Agriculture, Richmond, Va.

  b. ARSINE DISTILLATION AND GUTZEIT METHODS:
   Associate referee: C. R. Gross, Bureau of Chemistry and Soils, Washington, D. C.

 COPPER AND ZINC:
  Associate referee: R. M. Mehurin, Bureau of Animal Industry, Washington, D. C.

 FLUORINE:
  Associate referee: Dan Dahle, Food and Drug Adm., Washington, D. C.

 LEAD:
  Associate referee: M. Harris, Food and Drug Adm., Chicago, Ill.

FRUITS AND FRUIT PRODUCTS:
General referee: B. G. Hartmann, Food and Drug Adm., Washington, D. C.

 SOLUBLE SOLIDS:
  Associate referee: L. H. McRoberts, Food and Drug Adm., San Francisco, Calif.

 ASH:
  Associate referee: Doris Tilden, Food and Drug Adm., San Francisco, Calif.

 FRUIT ACIDS:
  Associate referee: B. G. Hartmann.

 EFFECT OF H-ION CONCENTRATION ON EXTRACTION OF FRUITS:
  Associate referee: L. A. Salinger, Food and Drug Adm., San Francisco, Calif.

 MOISTURE IN DRIED FRUIT:
  Associate referee: Paul Clifford, Food and Drug Adm., Washington, D. C.

CANNED FOODS:
General referee: V. B. Bonney, Food and Drug Adm., Washington, D. C.

VINEGARS:
General referee: A. M. Henry, Food and Drug Adm., Philadelphia, Pa.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:
General referee: J. B. Wilson, Food and Drug Adm., Washington, D. C.

MEATS AND MEAT PRODUCTS:
General referee: R. H. Kerr, Bureau of Animal Industry, Washington, D. C.

SEPARATION OF MEAT PROTEINS:
Associate referee: W. S. Ritchie, University of Missouri, Columbia, Mo.

GELATIN:
General referee: R. M. Mehurin, Bureau of Animal Industry, Washington, D. C.

CACAO PRODUCTS:
General referee: J. W. Sale, Food and Drug Adm., Washington, D. C.

MILK PROTEINS IN MILK CHOCOLATE
Associate referee: Marie L. Offutt, Food and Drug Adm., New York City.

CACAO BUTTER:
Associate referee: W. O. Winkler, Food and Drug Adm., Washington, D. C.

SUCROSE AND LACTOSE IN MILK CHOCOLATE:
Associate referee: J. Fitelson, Food and Drug Adm., New York City.

COFFEE:
General referee: E. M. Bailey, Agricultural Experiment Station, New Haven, Conn.

GUMS IN FOODS
General referee: L. J. Cross, Dept. of Dairy Ind., Agr. College, Ithaca, N. Y.

SPICES AND OTHER CONDIMENTS:
General referee: H. A. Lepper, Food and Drug Adm., Washington, D. C.

VOLATILE OIL IN SPICES:
Associate referee: J. F. Clevenger, Food and Drug Adm., New York City.

BAKING POWDERS AND BAKING CHEMICALS:
General referee: W. C. Geagley, Department of Agriculture, Lansing, Mich.

MICROCHEMICAL METHODS:
General referee: E. P. Clark, Bureau of Chemistry and Soils, Washington, D. C.

MICROBIOLOGICAL METHODS:
General referee: A. C. Hunter, Food and Drug Adm., Washington, D. C.

CEREAL FOODS:
General referee: J. A. LeClerc, Bureau of Chemistry and Soils, Washington, D. C.

ASH IN FLOUR, ALIMENTARY PASTE, AND BAKED PRODUCTS; CHLORIDE IN FLOUR AND BAKED PRODUCTS; AND COLOR IN FLOUR:
Associate referee: D. A. Coleman, Bureau of Agricultural Economics, Washington, D. C.

H-ION CONCENTRATION OF FLOUR:
>   *Associate referee*: Rowland J. Clark, Schulze Baking Co., Kansas City, Mo.

DIASTATIC VALUE OF FLOUR:
>   *Associate referee:* M. J. Blish, Agricultural Experiment Station, Lincoln, Nebr.

STARCH IN FLOUR:
>   *Associate referee:* Lewellyn Jones, Food and Drug Adm., Kansas City, Mo.

FLOUR-BLEACHING CHEMICALS:
>   *Associate referee:* Dorothy Scott, Food and Drug Adm., New York City.

FOREIGN METHODS FOR TESTING FLOUR:
>   *Associate referee:* C. H. Bailey, University of Minnesota, Minneapolis, Minn.

CO_2 IN SELF-RISING FLOUR:
>   *Associate referee:* L. D. Whiting, Ballard and Ballard, Louisville, Ky.

SAMPLING ALIMENTARY PASTES AND DETERMINATION OF MOISTURE IN BAKED PRODUCTS:
>   *Associate referee:* L. H. Bailey, Bureau of Chemistry and Soils, Washington, D. C.

CRUDE FIBER IN BAKED PRODUCTS:
>   *Associate referee:* Ruth G. Capen, Bureau of Chemistry and Soils, Washington, D. C.

METHODS FOR FLOUR, ALIMENTARY PASTES, AND BAKED PRODUCTS:
>   a. UNSAPONIFIABLE MATTER:
>   >   *Associate referee:* S. Alfend.
>
>   b. FAT, CRUDE ALBUMIN NITROGEN, LIPOIDS, AND P_2O_5
>   >   *Associate referee:* L. C. Mitchell, Food and Drug Adm., St. Louis, Mo.

MILK SOLIDS IN MILK BREAD:
>   *Associate referee:* Arnold Johnson, 1403 Eutaw Place, Baltimore, Md.

RYE IN FLOUR MIXTURES:
>   *Associate referee:* J. H. Bornmann, Food and Drug Adm., Chicago, Ill.

EXPERIMENTAL BAKING TESTS:
>   *Associate referee:* C. G. Harrel, Pillsbury Flour Mills Co., Minneapolis, Minn.

BEERS, WINES, AND DISTILLED LIQUORS:
>   *General referee:* W. V. Linder, Bureau of Industrial Alcohol, Washington, D. C.

MEMBERS AND VISITORS PRESENT,
1932 MEETING

Adams, W. L., Agricultural Experiment Station, Kingston, R. I.
Allen, C. E., Experimental Farm, Ottawa, Canada.
Allison, F. E., Bureau of Chemistry and Soils, Washington, D. C.

Bacon, C. W., Bureau of Plant Industry, Washington, D. C.
Badger, C. H., Food and Drug Administration, Washington, D. C.
Bailey, E. M., Agricultural Experiment Station, New Haven, Conn.
Bailey, L. H., Bureau of Chemistry and Soils, Washington, D. C.
Balls, A. K., Bureau of Chemistry and Soils, Washington, D. C.
Balthis, T. A., Department of Agriculture, Richmond, Va.
Barbella, N. G., Bureau of Animal Industry, Washington, D. C.
Barnard, H. E., Corn Industries Research Foundation, Indianapolis, Ind.
Bates, F., U. S. Bureau of Standards, Washington, D. C.
Batton, H. C., Swift and Company, Baltimore, Md.
Beal, W. H., Office of Experiment Stations, Washington, D. C.
Beebe, C. W., Bureau of Chemistry and Soils, Washington, D. C.
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WILEY MEMORIAL LECTURE. NO. II

CHEMICAL STIMULANTS AND THEIR EFFECT UPON THE GROWTH AND METABOLISM OF DORMANT PLANTS[1]

By F. E. Denny (Boyce Thompson Institute for Plant Research, Yonkers, N. Y.)

I am glad to contribute my bit toward maintaining the custom of devoting this hour as a memorial to Doctor Wiley, and I think that this plan of having an invited speaker will be even more effective than was hoped for by those who instituted it. For, as the years pass by and as the invited speakers come and go, it will be brought home to all of you each year how impossible it is for any speaker on your program to take the place of Doctor Wiley. He was a master of a multiplicity of themes. Those of you who listened to him during that remarkable series of talks will remember upon what widely different topics he spoke; at one time or another on astronomy, oysters, bolshevism, chaos, bleaching of flour, wooden nutmegs, permanent waves, marriage, and oligonumismaty (this last word being one of his own invention and which means "shy of coin"). Such topics are not for speakers who are to succeed him here. Moreover, Doctor Wiley was able to speak either seriously or humorously upon any of these topics, and even in both ways in the same sentence. But for those of us who are to come later to speak seriously upon any of these topics would be humorous, and to speak humorously of them would be serious indeed. No, we are not going to be able to take Doctor Wiley's place at these meetings, but we are going to be proud to be here and to demonstrate by our smallness how really great he was.

The subject of the present discussion seems appropriate for this occasion because it relates to a field in which Doctor Wiley took a very lively interest, namely, the effect of chemicals upon the life activities of plants. Of course, only a particular portion of this field is dealt with in this paper.

That many species of plants have periods of rest during which the buds cannot grow; that small amounts of chemicals, which are non-nutritive and which the plant does not encounter in nature, can break this dormancy and induce bud-growth; that in doing this the chemicals stimulate many of the processes of metabolism in the tissue—these are the principal points to be discussed in this paper.

We deal here not with chemicals which influence the growth of buds that can grow or are already growing; we deal with chemicals which can initiate growth in buds that under ordinary conditions cannot grow at all.

In these species with dormant periods, as active growth proceeds the

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plant forms buds that are to continue the growth in the succeeding period. These buds become mature at the end of the growing season, and under usual cultural methods do not start at once upon another cycle of growth. The plants are then in what is called the rest or dormant period. But the reader should understand that these are merely terms of convenience; we do not know that the plants are "sleeping" or that they "rest"; we merely mean that in this stage of the life cycle the buds do not grow under the conditions of moisture, temperature, and nutrients that ordinarily induce a rapid growth.

As examples of plants which have rest periods we may mention potatoes, various bulbs such as gladiolus, and nearly all woody plants such as lilac, apple, plum, etc. Potato tubers from a newly harvested crop when planted again will not grow; only after a rest period of two to three months can good germination be obtained. Gladiolus bulbs harvested when the tops die in September and planted at once, will not grow until about December or January, or with some varieties, even later. Lilac plants, if brought indoors as soon as the leaves fall, will remain for many weeks with unopened buds even if the conditions ordinarily used for early forcing of these plants are maintained continuously.

The rest period of such plants may be broken by the use of chemicals, and an early or nearly immediate growth of buds can be obtained, even when the treatments are carried out upon plants in their most deeply dormant period. That chemicals could be used for this purpose was shown first by Johannsen (16) who used ethyl ether with good success in the case of woody plants such as lilac, and less successfully with potatoes and bulbs. Later, among the chemicals tested McCallum (18) found that ethyl bromide, ethylene dichloride, and carbon tetrachloride had good forcing effects upon dormant potatoes. These and many other chemicals were tested in experiments which were started in 1924 and which have continued until the present time. Also, studies have been made regarding the effects of these chemicals upon the respiration, enzyme activity, and other phases of the metabolism of the plants. The object of this paper is to discuss these results and to describe the present status of the problem.

FAVORABLE METHODS OF TREATMENT WITH CHEMICALS

To assist the reader in understanding this discussion, the following brief description is given of the most successful chemical procedures that have been found at present for breaking the dormancy of potatoes, gladiolus, and lilacs (2, 3, 9).

Potatoes.—Two different methods may be used. (a) The tubers are cut into one-eye pieces weighing about 25 grams. These pieces are put in a Mason jar and are covered with a solution of 50 cc. of 40 per cent ethylene chlorhydrin in a liter of water. The solution is poured off at once and the jar is inverted to facilitate removing the excess liquid by shaking. This

leaves a film of the liquid on the potatoes and on the inside of the jar. The jar is closed and is placed at room temperature (not over about 30° C.) for 24 hours. Then the potatoes are removed and planted. (b) The tubers, cut as described above, are soaked for 1.5 hours in a solution of 10 grams of sodium thiocyanate in a liter of water. The treated potatoes are planted without removing the film of chemical that adheres to the surface.

Gladiolus.—After removal of the outer brown husk the bulbs are put in a jar of about 4 liters capacity. Then 10 cc. of 40 per cent ethylene chlorhydrin are taken up in a piece of cheesecloth of the proper size to prevent dripping. After the cheesecloth has been laid on top of the bulbs the jar is sealed. If the jar does not have a tight cover it can be sealed sufficiently well by the use of artists' clay. After two days the bulbs are removed and are planted in soil.

Lilac.—Potted plants, two to four years old, are placed in a 4000 liter galvanized iron container which is practically air-tight and in which cheesecloth which has absorbed 320 cc. of 40 per cent ethylene chlorhydrin is suspended from the ceiling. Evaporation from the cloth is assisted by circulating the air inside the container by means of an electric fan. After 22 hours the plants are removed and placed in the greenhouse.

These procedures have been tested for several years with uniformly favorable results. Some varieties of gladiolus respond less readily than others, but even with the more difficult types good germination has been obtained at some later stage of the rest period when the germination of freshly-harvested bulbs was forced only partly.

The gains in the time of sprouting of treated and check lots have varied in different experiments with different kinds of plants at different stages of dormancy. The details may be found in the complete reports. In general, gains of about 20 days are common, and 40 days are not unusual if the treatments are applied early in the rest period.

EFFECTIVENESS OF DIFFERENT CHEMICALS

In making comparisons of the effectiveness of chemicals the following factors should be taken into consideration: (a) Stage of the rest period at which the treatment is applied. As the plants progress through the rest period they become more and more susceptible to the influence of the chemical. Hence a chemical which will give a good result toward the end of the rest period may be quite ineffective if applied in the period of deepest dormancy. For example, sodium nitrate can induce early germination of potato tubers if it is applied late in the dormant period but such a treatment is unsuccessful with freshly-harvested tubers; sodium thiocyanate, on the other hand, can be used with tubers from plants that have just matured their crop. (b) The concentration required for a forcing effect. If the required concentration is too high the cost may be too great or the inconvenience of applying such high concentrations may make the

method impractical. This is the case with ethyl ether and ethylene. Lower concentrations of such chemicals may be used if the time of application is increased, but this merely introduces another difficulty. (c) Range of concentration over which a successful result can be obtained. Thus, carbon tetrachloride has a narrow range, while that for ethylene chlorhydrin is wide. (d) Promptness with which growth will begin after the treatment has been applied. This has been a less important factor as the chemicals usually either induce prompt growth or fail completely. However, in the case of potatoes, although both ethylene chlorhydrin and sodium thiocyanate will hasten sprouting, this occurs usually about four to seven days later with thiocyanate than with chlorhydrin. (e) Application to different species and to different varieties of the same species. Sodium thiocyanate is effective with potatoes but not with gladiolus nor lilac, while ethylene chlorhydrin is effective with all three species. (f) Safety from explosions and toxicity in applying the treatments. The use of ethyl ether and hydrogen cyanide (10) is very dangerous in these respects. Although ethylene chlorhydrin vapors do not produce an explosive mixture when combined with air, it has been found that when breathed for a rather long period its vapors in high concentration are toxic to humans, and deaths have resulted in England and Germany in connection with the manufacture of the chemical and its use as a solvent in the preparation of dyes (19). Ethylene dichloride vapors are combustible but if mixed with carbon tetrachloride in the ratio of 3 to 1 by volume the mixture gives a non-explosive vapor. This mixture has been tested with plants and is quite effective in breaking dormancy, but it is likely that its vapors could become toxic to humans under certain conditions.

Ethylene chlorhydrin is, at present, the most promising chemical, as it has been applied successfully to more different kinds of plants, in more different stages of the rest period, and within a wider range of concentration than has any other chemical tested. Other chemicals that usually give successful forcing are ethylene dichloride (either alone or mixed with carbon tetrachloride), ethyl bromide, and carbon bisulfide. With potatoes especially favorable results are obtained with the thiocyanates of sodium, potassium, ammonium, or calcium, and with thiourea.

It will be noted that some of these compounds have sulfur as a constituent. It has been found (20) that many sulfur-containing compounds, such as ammonium dithiocarbamate, thiosemicarbazide, hydrogen sulfide, ethyl mercaptan, etc., show excellent forcing effects with potato.

EFFECT UPON METABOLISM

In studying this phase of the subject, treated and check lots were compared as to respiration, enzyme activity, chemical composition, etc., samples being taken at intervals after the treatment but before visible growth of buds occurred. In some experiments the concentrations of the chemical

formed a decreasing series, and in this way the influence of the chemical at the optimum and also at suboptimal concentrations could be studied.

For a detailed description of the results the original articles should be consulted (8, 12, 14), but some of these results are discussed in the following paragraphs.

Respiration.—The respiration effects have been measured for lilacs and potatoes (7, 22). In the case of lilacs the rate of production of carbon dioxide was increased by treatment with ethylene chlorhydrin vapors by an amount which varied in different experiments from about 10 per cent to as much as 200 per cent. With potatoes, if whole tubers were used, the increases were 300–700 per cent; but if cut tubers were used, the increases, although still present, were much smaller. A factor reducing the magnitude of the difference between treated and check lots of cut tubers is the large increase in the respiration of the check lots as a result of merely cutting the tuber.

These respiration increases are initiated in the tissues shortly after the chemical treatments are applied, and in some cases they can be observed within 24 hours.

In general it may be stated that treatments that were successful in hastening growth also increased respiration; but the possible corollary of this, that whatever increases respiration also hastens growth, is not true; neither storing at a low temperature for a few hours (which leads subsequently to much higher respiration) nor increasing the temperature (which may increase the respiration two- to three-fold) has any marked favorable effect upon the growth of the buds. The increase in respiration obtained when potato tissue is cut may be explained in part by the effect of cutting in facilitating the entrance and exit of gases. But the large increases in respiration induced in intact tissues by the treatment with chemicals is probably not due merely to an increase in the permeability of the tissue to gases. Probably the oxidizing and reducing systems play a part here, and it is shown in a subsequent paragraph that large increases in the activity of these systems are produced by the chemical treatments.

Enzymes.—In most cases the catalase activity was increased by the chemical treatments, to a great extent by ethylene chlorhydrin, and less effectively by other chemicals. A similar situation was found with peroxidase and with the capacity of the tissue to reduce methylene blue. It should be emphasized that these increases in enzyme activity are not due to a direct effect of the chemical upon the enzyme. If the chemicals are added to press-juice it is found that the chemicals either retard enzyme activity or have little effect upon it. These enzyme increases are obtained only by treating the living tissue with the chemicals and testing for enzyme activity at a later period, after the chemicals have had time to induce a response in the tissue.

The amylase and invertase are likewise increased by the treatments,

although the influence varies with the kind of plant and with the chemical used. The suggestion has been made (1,17) that the favorable effect of sodium thiocyanate upon the germination of potatoes might be related to the direct effect of the chemical in hastening amylase activity, as it has been shown that small amounts of thiocyanate increase the starch-splitting power of saliva, malt, and takadiastase. With potato juice, however, tests show (5) that thiocyanate does not hasten amylase activity; it retards it. But if potato tissue is treated with thiocyanate the press-juice obtained at a later period will be found to have higher amylase activity. The chemical effect is not a direct one upon the enzyme; it is upon the living matter which, in turn, influences the amount of (or the activity of) the enzyme (6).

Sugars.—Since sugars furnish energy and are an important source of building material for growth, it might be expected that increased amounts of sugars would be built up in the tissues preceding the initiation of growth, and that a correlation between sugar content and bud development might be found.

The early measurements with potato were hopeful for this point of view, as sucrose values in the treated tissues showed increases and formed a series which was correlated well with the series of concentrations of the chemical used in the treatment, and with the subsequent growth of sprouts (4). Later measurements, however, of the sugars in treated and check lots of gladiolus (unpublished) and lilac (7) show that the relationship is not as simple as this, and that along with the rate at which higher carbohydrates are broken down into simpler forms, two other factors are operating to influence the amount of sugar that may be found in the tissue at any sampling period; namely, the supply of available higher carbohydrates (which influences the amount of sugars that may be formed) and the tissue respiration (which influences the rate at which the sugar disappears). The analytical results that are obtained at any stage of the process are merely a measurement of the state of this equilibrium at the time of sampling. The data are in favor of the view that it is not the amount of sugar present that is the important factor, but it is the capacity of the tissue to produce it and to utilize it.

Glutathione.—In connection with the effect of chemical treatments upon the reductase in potato and gladiolus it was found that a non-enzymic reducing system was present, which reduced iodine to substances not giving a blue color with starch, and reduced sulfur to hydrogen sulfide. The activity was lessened by the presence of oxidase (11) and it is preferable to study this system in boiled juice. Further work showed that two types of substances are involved in this effect: (a) Sulphydryl compounds which reduce both iodine and sulfur; (b) a non-sulphydryl component (possibly similar to or identical with hexuronic acid) which reduces iodine but not sulfur. In potato the principal sulphydryl component is glutathione, but

that in gladiolus has not been identified yet, although it is probably glutathione. An improved method of estimating glutathione based upon this capacity to reduce sulfur to hydrogen sulfide has been developed (15); the iodine titration method for glutathione gives results that are too high if these non-sulphydryl iodine-reducing substances are present. Treatment of potato and gladiolus with ethylene chlorhydrin greatly increases the content of the tissue in these sulfur-reducing substances. This effect is shown especially well in the case of potato. Hopkins' method for isolating glutathione when applied to potato tubers that have been treated previously with ethylene chlorhydrin gave a large precipitate of the cuprous salt from which crystalline glutathione was obtained (13). The same procedure with juice from untreated tubers did not yield a precipitate, and no glutathione could be obtained, although qualitative tests indicated that a small quantity was present.

pH.—Potato juice usually has a *p*H of about 6.0, but if the tubers are treated with vapors of ethylene chlorhydrin the juice obtained from them after about two days will have a pH value of 6.5 to 7.0. Since potato juice is strongly buffered it will be realized that an extensive change in the juice would be necessary to permit this shift in reaction. Solutions of ethylene chlorhydrin are slightly acid, while the change produced in the potato is in the alkaline direction. The change, therefore, cannot be a direct effect of the chemical; it is brought about by an indirect effect in influencing the metabolism of the living tissue (21). It does not seem that this change in *p*H is a critical one for the initiation of sprouting, because other chemicals, such as sodium thiocyanate or thiourea, induce sprouting without causing any large shift in the *p*H value, and ethylene chlorhydrin induces sprouting in gladiolus although the *p*H change which results is small. However, apart from any question of sprouting, this result emphasizes the powerful influence which the vapors of a chemical can have upon the cell activities which produce the mixture of buffer substances that determines the *p*H value.

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PRESIDENT'S ADDRESS[1]

CONSERVATIVE PROGRESSIVENESS

By ARTHUR E. PAUL (U. S. Food and Drug Administration, Chicago, Ill.)

Were the question asked, what constitutes the most fundamental doctrine of the Association of Official Agricultural Chemists, the reply would be, "Conservatism." Conservatism is the tendency to preserve in a safe or entire state that which has been established. It opposes taking actions without full consideration as to safety and desirability. Conservatism provides stability, but it also permits action with energy and vim equal to that engendered by enthusiasm. Conservatism is the opposite of hastiness, but it is not the opposite of progressiveness; in fact it increases the effectiveness and value of progressiveness. Progressiveness is an urge to move forward; it does not imply hastiness, neither does it necessarily exclude this tendency. It is a desirable quality, provided there is coupled with it a restraining and directing influence to assure movement in the right direction and along desirable lines. Progressiveness may be impetuous, reckless, and rash, or it may be careful, cautious, and sound. A tendency toward conservatism may be inherited, but conservatism itself is manifested only as experience teaches the effects of recklessness and rashness. An infant will creep into serious danger without any consideration of the disastrous results which may follow. With accumulated experience the need is learned for considering beforehand the results of any steps which are taken. Conservatism develops maturity. With natural timidity and strong inherited conservatism, there may result an excessive conservatism, with a resultant impairment of progressiveness and a consequent impairment of efficiency and advance.

To the extent that the qualities of progressiveness and conservatism may be consciously and intentionally controlled, we should in all our undertakings attempt to preserve a proper balance between them. We must foster progressiveness by all means, but we must exercise conservatism in working toward our goal of maximum success.

Let us consider the relative tendencies of our Association toward these two fundamentals, progressiveness and conservatism.

BUSINESS

This Association is not primarily a business enterprise, and yet there are involved strictly business features. Payments are received, and disbursements are made. This association is perhaps the only organization to which any one of us belongs which has during war-time and peace-time, during years of prosperity and in periods of depression, never solicited

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contributions. The only payment that members make is the subscription price of *The Journal*, or of *Methods of Analysis*, both practically at the actual cost of publication. And yet, our treasury is in splendid condition, due largely to the efficiency, farsightedness and unselfishness of our financial officers, and also to the fact that our progressive ventures are tempered with conservatism.

PUBLICATIONS

In the early years of our Association, the proceedings were published by the United States Department of Agriculture, and likewise, the first few editions of the methods adopted were printed under one cover by that same Department. During that early period our Association published nothing. However, it showed an abundance of progressive enterprise when, in 1917, it entered the publishing field, launching the now well-known *Journal of the Association of Official Agricultural Chemists*, which has grown steadily in quality and scope. At the present time our *Journal* includes proceedings of our conventions, and in addition many splendid and original contributed scientific articles. In connection with these publishing ventures our Association maintains an editorial staff, which carefully, rigidly, and conservatively reviews all articles and items before they are accepted.

As publishers, therefore, we have shown marked progressiveness in undertaking new ventures. At present we also publish *Methods of Analysis*, which is one of the most important books found in any library. No official food, drug, or other agricultural laboratory could carry on without this book, and it is recognized as an authority the world over.

Further evidence of the association's progressiveness is the re-editing of Wiley's "Principles and Practice of Agricultural Analysis." The task of rewriting this work was undertaken by some of the members of this association. Two of the three contemplated volumes have already been published, and the third, it is hoped, will appear in the near future.

The plan is that Volume 3 shall be a companion book to our *Methods of Analysis* and that it shall contain authentic analyses and data which will enable the interpretation of results obtained by our official and tentative methods. There is a need for this book, and it is hoped that our association will complete the task of publishing and marketing this third volume.

METHODS OF ANALYSIS

The principal activity of the Association of Official Agricultural Chemists is the study, development, thorough investigation, and adoption of methods for the examination of agricultural products. The general procedure employed in connection with the formulation of these new methods is familiar to the older members present and will soon be well understood by the newer members.

If we analyze the process, we shall see that both progressiveness and conservatism must function freely. The referees, associate referees, and collaborators, who initiate and develop these new methods, must, primarily, possess progressiveness. They must exercise activity, enterprise, and originality. It is necessary that they plan and do new things, and that they do them promptly. However, with respect to the adoption of these methods, our association has been inflexible in its conservatism. The work is reviewed by the respective committees and finally by the Association in its convention. With this combination—progressiveness in the development of methods and conservatism in their adoption,—the growth in scope and importance of this association has been inevitable.

While the work of the individual is subject to review, he must, nevertheless, assume unlimited personal responsibility. The work of the referees and associate referees should be complete and correct before it leaves their hands. The subsequent reviewers cannot complete nor materially alter the report; they can approve it, remand it for further study, or reject it.

The collaborators' work is outlined for them, it is true. But conclusions as to the accuracy of a method is based upon the results obtained by these collaborators, and any inaccuracies in their work reflect against the methods.

While, therefore, the mechanism of the preparation and adoption of methods is complex, and while there is a maximum of conservative review, there is not only ample opportunity, but rather absolute necessity, on the part of each individual, to assume personal responsibility.

SCOPE

The name of this Association would indicate that our membership is restricted to chemists and our field to purely chemical methods. However the Executive Committee, last year, amended the constitution so as to include bacteriologists, microanalysts, and other scientists. This action very materially liberalizes the permissible scope of our activities.

As a matter of fact, our Association has not been excessively conservative in its willingness to take up new topics. I might refer to Dr. C. A. Browne's most timely and constructive address at last year's meeting and to the talks by Dr. Balls and Dr. Phillips which followed, as a result of which this association entered the new field of enzymes and also that of the study of the composition of lignin.

Another item of special interest developed in the drug section during the last two years by Dr. Arno Viehoever, of the Philadelphia College of Pharmacy, may prove to be an epoch-making accomplishment in the field of biological testing. The method now in vogue of observing the effect of the substance under examination upon an animal of relatively high order or upon one of its organs, as for example, guinea pigs, cats, dogs, frogs, rabbits, or chickens, is time-consuming, costly, and uncertain

because of the animal's possible idiosyncrasies. Dr. Viehoever employs, instead, a small infusorian and observes the effect at once, microscopically. He uses a large number of these organisms, thereby eliminating their individual idiosyncrasies. He has found that without exception his results agree perfectly with those obtained through carefully checked findings on the higher animals.

Originally, the purpose of this organization was the formulation and adoption of uniform methods of analysis for fertilizer control. Subsequent to the unification of these methods, consideration was given to soils, plant constituents, insecticides, leather, tanning materials, and waters. Feeding stuffs followed and served as a stepping stone to the now very important series of food subjects which take up nearly one-half of *Methods of Analysis*. Because of the importance of the methods of this Association to the officials enforcing Federal, State, and City food control laws, which also include drugs, this subject was taken up a few years ago. As seen from this list, there is a continual broadening of the field, the subjects taken up being less and less directly agricultural in their nature, yet in each case definitely related thereto. The latest subjects to be included are naval stores and paints.

ABBREVIATED METHODS

In connection with drugs, there is a further agency which merits our careful attention. It is known as the Combined Pharmaceutical Contact Committee, and it includes representatives of the American Drug Manufacturers' Association and the American Pharmaceutical Manufacturers' Association. It is the function of this Contact Committee to establish permissible tolerances or variations in composition, and to devise brief methods for the examination of pharmaceutical preparations that are especially adapted to factory control. Many of these methods have been prepared by the Pharmaceutical Contact Committee, and promulgated by the Food and Drug Administration of the United States Department of Agriculture. Manifestly, for factory control purposes abbreviated methods may be used. The manufacturer knows the exact ingredients used and knows the presence or absence of interfering substances.

For use by public officials, too, abbreviated methods are frequently of service. In several cases our Association has already seen fit to adopt this type of procedure. For instance, the Gutzeit arsenic method, which was originally adopted as a preliminary method, to be checked by the older, far more difficult and time-consuming Marsh method, has been so thoroughly studied and modified by this Association that it now yields results astonishingly close to the truth.

The Babcock method for the determination of fat in milk also represents a brief procedure, which, in case of contested court action, should no doubt be confirmed by the more exact extraction method.

In the case of coal tar dyes in foods, we also have in the 1930 edition of *Methods of Analysis* an abridged process for their separation.

Other examples might be cited, and still others will present themselves. The question, then, that arises is whether it will be our policy to favor the study and the adoption of abbreviated methods, in addition to our more complete and perfect procedures, or whether we shall discourage this idea and restrict our activities as much as possible to complete methods. Progressiveness may point one way, conservatism the other. The question merits careful consideration.

RELATIONS WITH OTHER ORGANIZATIONS

A number of other organizations concern themselves with analytical methods and we must, in connection with our own activities and our own future, give careful consideration to our relationship with them.

The United States Pharmacopoeia is the accepted authority on drugs and includes methods for the examination of many drug products. It is revised every ten years. Careful investigations are made before the methods are adopted for inclusion in the revisions. It has been the policy of the Association of Official Agricultural Chemists to consider the Pharmacopocia methods as final and satisfactory unless experience shows that there is a possibility of improvement. In the case of products for which the Pharmacopocia has no methods, our Association undertakes studies to develop suitable methods. If, subsequently, our methods are adopted by the Pharmacopoeia, then they are eliminated from our tentative or official list and omitted from our *Methods of Analysis.*

The American Chemical Society issues a quarterly analytical edition which includes agricultural products. However, it assumes no particular responsibility for the methods published. A number of trade organizations, as, for example, the Cottonseed Crushers Association, the Insecticide and Disinfectant Manufacturers Association, the National Canners' Association, actually study methods, but they usually adopt and utilize our official methods. In fact, it seems that this organization and that of the United States Pharmacopoeia are about the only ones that make a special study of analytical methods, adopt them, and then assume responsibility for their correctness and reliability.

The Federal Public Health Service studies, devises, and adopts methods. While these methods cover a special field, there is more or less overlapping, and possibly we may be able to maintain closer cooperation with that organization than has been done in the past.

The United States Army, Navy, and other departments, as well as many States and Cities, purchase supplies and other needs on specifications, and these, at times, involve methods of examination. These departments, however, usually refer to the methods of our organization whenever they are applicable. Trade organizations, too, while interested

in methods, usually follow those promulgated by the Association of Official Agricultural Chemists.

We must attempt to observe our Association in its setting among these other organizations in order that we may best decide how to derive a maximum of benefit from our contact with them, and that we, in turn, may be of maximum service to them. Furthermore, we should avoid any duplications by informing ourselves as fully as practicable as to the activities of these other organizations. Let us, therefore, consider establishing contacts, wherever this has not yet been done, through the appointment of committees or individuals, who will attend the meetings of these agencies, or, if this is impossible, will correspond with their secretaries. Reports may then be submitted at our conventions for consideration along with reports on other activities of our Association.

CONCLUSIONS

From these considerations we find that while this organization is in all its diversified activities, and in all its aspects, eminently conservative, it is also decidedly progressive. It is progressive in its business affairs and commendably conservative in its expenditures. It has been decidedly progressive in the matter of entering new fields, in taking up new subjects, and in studying new methods; but it is unique in its conservatism in finally adopting methods. The requirements are so rigid that any danger of erroneous methods being included is practically excluded. No other agency in this or any other country, so far as we are aware, parallels it, and as a result it merits the unique distinction of authority which is accredited to it by the chemical world. Its methods are generally accepted and adopted everywhere without question. It must be concluded that this type of conservatism in the adoption of methods should be rigidly continued. If, upon careful conservative consideration, it is found advantageous to adopt additional short-cut methods, applicable in special instances, its tendencies toward progressiveness will unduce the association to do this. So, too, if additional contacts with other organizations or agencies are found to be desirable, then the indicated steps will follow after careful, conservative, yet progressive, deliberation.

Our Association includes members of all ages and of widely different dispositions and inclinations; some lean toward progressiveness, others toward conservatism. The combination of these divergent tendencies forms this truly productive association. As the years roll on, as officers come and officers go, as committees succeed committees, and as the older members necessarily relinquish their more strenuous duties and ultimately disappear, the younger members will acquire conservatism in addition to their progressiveness and will take their turn to become the older members. It is to these younger members that I wish to address my closing remarks. There is work for all of you. If you are asked to serve in

any capacity, be it as collaborator, as referee, or as associate referee, or whatsoever it may be, remember that this is your Association and that it needs your support. Accept the service, and in so doing give it your best efforts. Assume personal responsibility for the completeness and correctness of your work. Render a report of which you will be justly proud. I advise that if you should be overlooked and not asked to accept a definite assignment, you show your progressiveness by volunteering your services. Everyone is endowed with qualifications in which he especially excels, and these talents can be applied and dedicated to the public welfare.

The accomplishments of our organization reflect the achievements of those individuals who have contributed to it in the past, and the future responsibility of carrying on the work to higher and higher planes of perfection must ultimately be entrusted to you. Will you not, therefore, express to any officer, either during this convention or in writing at any time your willingness to serve? Thus you will be in a position to lend your influence and assistance in perpetuating the principles of conservative progressiveness which have carried this organization through nearly half a century of success and growth, and assure for future generations that its permanent watchword will continue to be:

"Progressiveness where progressiveness is needed;

Conservatismwhere conservatism should be heeded."

ORDER OF PUBLICATION

The reports of the committees presented on the last day of the annual meeting are given at the beginning of the proceedings, not in their chronological order. This arrangement will assist the referees, associate referees and collaborators in planning and developing their year's work. The remainder of the proceedings will then follow in the usual order.

THIRD YEAR

WEDNESDAY—AFTERNOON SESSION
REPORT OF EDITORIAL BOARD

The financial statement will show how successfully most of our publication work is now functioning.

The plan adopted some years ago of consolidating the editorial activities under one administrative board has proved quite satisfactory. The policy of inviting the chairmen of the three editorial committees to meet with the Executive Committee at its annual meeting has also proved to be a desirable innovation. We are endeavoring to bring together under the Executive Committee all the activities of the Association.

As you may remember, last year I made a request of the Executive Committee for an appropriation not to exceed $1,000 for the purpose of making an index of all the publications issued by the Association. Of this sum, $465.00 has been spent. The index is about two-thirds completed. It was necessary to engage additional help in Miss Lapp's office, a young lady for part of the year, to allow Miss Bates and Miss Lapp to pay attention to the indexing. It is proving to be a laborious task, but when it is completed under the plans outlined we shall have a complete index of everything undertaken by the Association from its organization down to 1930. We shall then have an index prepared every 10 years, coincident with our edition of *Methods of Analysis*.

Dr. Browne will tell you in detail about the difficulties we are having with the distribution of Volume II of the Wiley book. This matter was discussed at the Executive Committee meeting. It may be necessary for the Association to take over the volumes that are now in the hands of the printer. The Executive Committee also authorized the distribution of a single copy to each contributor to Volume II. We are in a rather peculiar position because the real ownership of the thousand copies rests with the publishing company until the cost of publication is liquidated. Not until then do we begin to obtain any royalty. At this time I am rather apprehensive whether any royalty can be obtained for several years.

At this time the representatives of the three editorial committees will give separate reports.

W. W. SKINNER, *Chairman*

Approved.

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REPORT OF THE EDITORIAL COMMITTEE OF THE JOURNAL

The Editorial Committee of *The Journal* held a meeting just prior to the sessions this year and prepared this report. It has been advised that the finances of the Association so far have not reflected the present economic situation. In other words, if we don't lose any more subscriptions we shall not have cause to worry.

The circulation of *The Journal* at the present time is 582 copies, domestic, and 242 copies, foreign, or a total of 824 copies. There has been a loss of 15 domestic subscriptions and a gain of 5 foreign, or a net loss of 10. There are 13 domestic and foreign complimentary copies and 19 exchanges. In volume 15, there are 688 pages plus the pages devoted to obituaries, contents, etc., or a total of over 700 pages. The contributed papers this year represent 215 pages. The average of contributed papers for the three previous volumes is 122 pages. The gain this year is 93 pages of contributed papers, which represents almost the gain in pagination of *The Journal*. We have not only increased the number of contributed papers, but they have been uniformly of a high standard of excellence.

There has been a loss of two pages of advertising—that formerly purchased by the Central Scientific Company and the Arthur P. Thomas Company.

The Committee decided to continue the present policy of publishing *The Journal* without editorials chiefly because the infrequency of the publication does not lend itself to an editorial policy. The same is true with abstracts, and no attempt will be made to include a section on abstracts for the present.

The suggestion was made that reprints be cut down in size uniform with reprints of other technical journals. Since the margins of the journal will permit this, the committee voted that this be done, provided it will involve no prohibited cost.

A suggestion was made to the committee that a section of *The Journal* be set apart for letters of criticism, either favorable or adverse, of methods presented, pointing out that there has been hesitation in discussing papers presented at the annual meetings. The committee, however, decided that criticism of papers and methods was not only amply provided for by the annual meeting but should certainly be a feature of these meetings, as was pointed out by Dr. Browne and Dr. Fraps. The committee vetoed the suggestion for publishing letters of criticism and comment in *The Journal*. It was decided, however, to feature special articles from time to time relating either to official methods or to those provisional or new methods under consideration. These articles are intended to be a critical study of all methods relating to particular items; for example, organic acids were mentioned, particularly citric acid. A compilation of methods on the determination of iodine was another subject; possibly fluorine would lend itself to this feature. It is the intention to have these articles contributed

by author or authors selected by the Editorial Board, and the articles shall relate primarily and essentially to analytical procedure.

By W. S. FRISBIE for the Committee
F. C. BLANCK   R. B. DEEMER
H. R. KRAYBILL   C. D. HOWARD

Approved.

REPORT OF THE EDITORIAL COMMITTEE
OF METHODS OF ANALYSIS

The third edition of *Methods of Analysis* has now been in the hands of the members of this association and of the public for one year. During that time the editors have received not only favorable comments on the part of the reviewers but likewise numerous letters calling attention to certain errors, of which approximately fifty have been noted. A list of these errata appears in the November number of *The Journal*. In view of the fact that over 25,000 abbreviations which were not in the second appear in the third edition, not to mention the very numerous changes which have been made in the methods since 1925, it is hoped that the editors will not be too severely criticised because of the apparently large number of errors. These corrections are being sent to every purchaser of the book.

I am sure you will be glad to hear some of the comments of the reviewers. Here are a few of them:

The Analyst.—This book merits the highest praise. The work can be accepted as authoritative in the true sense of the word, the methods being up-to-date and reliable.

J. State Medicine.—A book no food or agricultural chemist can afford to do without. The work has been skillfully planned.

J. S. Africa Chemical Inst.—The work amply fulfills its claims and covers the requirements of chemists engaged in agricultural chemistry and as such is strongly recommended.

R. Fresinius.—The official methods are of especial interest to the German chemist, as they contain very much of value and importance for him.

Food Industries.—The book is improved by the grouping of the chapters into food and non-food classifications.

Intern. Sugar Journal.—The book is unique in the literature of analytical chemistry. Chemists will turn to the various chapters for guidance with every confidence in the reliability of the procedures described.

Ind. Eng. Chem.—This new volume, in addition to covering more diversified subject matter, is an improvement over the 2nd edition in the convenient arrangement of the chapters and in the placing of chapter numbers at the top, and page numbers at the bottom of each page.

Oil, Paint & Drug Reporter.—This new compilation of the official methods fully reflects the progress that has been made.

Cereal Chemist.—The third Edition of the Book of Methods of the A.O.A.C. stands as a tribute to this Association. For the bakery chemist the chapters (listed) should be of consummate interest. The book reflects in every chapter the great amount of careful work which was done by the Editorial Committee in the compilation and organization of the text.

A German critic says it is regrettable that modern analytical processes, such as precipitations with organic complexes and potentiometric titrations have not yet been included. This criticism should bestir every referee to be ever on the lookout for more modern methods. Another foreign criticism is that during the past 12 years only works of American origin have been used. It is probably time that if this is true we also divorce ourselves from this form of provincialism.

Already two meetings have elapsed since the third edition was compiled. It behooves this association to begin thinking and planning for the next revision. I earnestly recommend that every referee be urged by the proper officers of this Association to study the chapters in which are found the subject matter of his refereeship and to be prepared at least one year before the next revision is ordered to recommend changes for the improvement of all methods which need revision. I want to recall at this time, that when the third edition was being revised many of the referees were unable, because of lack of time, to make more than a very hasty study of the chapters under their jurisdiction, and as a result fully one-third of the errata can be traced to this haste on the part of the referees. Certain chapters naturally need much more severe revision than do others. In fact, certain chapters in the present book are essentially the same as they were in the second edition. It is impossible to assume that during these intervening years no progress has been made along these lines. We cannot, therefore, begin too early to rouse an interest in this matter.

By J. A. LeClerc for the Committee
W. W. Skinner   L. E. Warren
J. W. Sale   Marian E. Lapp
                G. G. Frary

Approved.

C. A. Browne: Before reading my report I should like to say a word in regard to the criticism of one foreign reviewer which Dr. LeClerc mentioned to the effect that American contributors to our publication *Methods of Analysis* are ignoring processes that are being carried out in Europe. As a matter of fact I don't place much stress on that criticism. I think if the critic reviewed the text of *Methods of Analysis* very carefully he would find that we have given much credit to European analysts and made full use of European analyses.

REPORT OF THE EDITORIAL COMMITTEE OF PRINCIPLES AND PRACTICE OF AGRICULTURAL ANALYSIS

Since the last meeting of the association, when the revised edition of Volume II of Wiley's "Principles and Practice of Agricultural Analysis" was first placed upon the market, we have waited to determine the re-

ception which this volume would receive from the chemical public before determining what steps should be taken with reference to the publication of Volume III.

It must be very regretfully admitted that the sales of Volume II up to the present time have not been commensurate with our expectations. This is no doubt in large part due to the present period of financial depression, when the purchasing power of those who are interested in a new volume upon the analysis of fertilizers and insecticides is greatly reduced below the normal. The rather high price of the book, which is $10.00 to non-members but only $7.00 to members of the association, has also no doubt been an important factor in reducing the expected sales of the book, since the demand for the new edition of the association's *Methods of Analysis*, which sells for $5.00, has not been seriously affected. As far as the attitude of our own membership is concerned the Committee feels it necessary to conclude that the publication of other analytical works than the association's *Methods of Analysis* is not a financially remunerative enterprise. Only 23 copies of Volume II of "Principles and Practice" have been sold by the association at the reduced price of $7.00 to members; the sales by the publishers to non-members is not known but it cannot be large for they have done but little in the way of advertizing or circularizing.

Volume II of "Principles and Practice" has met with a large number of favorable reviews both at home and abroad, and a selection of these has been assembled by Miss Lapp together with a table of contents of the book to be printed as a circular for distribution among interested chemists. Copies of this circular are available at this meeting and we are hoping that their wide distribution will result in an increased demand for the book. If these efforts fail to bring forth the desired response it would seem necessary to abandon the plan of publishing Volume III of the "Principles and Practice" for which manuscripts of twenty-two of the contemplated thirty-six chapters have been submitted. It is estimated that Volume III will have about twice as many pages as Volume II, which will necessitate its publication in two parts. The increased selling price required for financing such a work necessitates additional caution at a time when both publishers and prospective purchasers of expensive scientific books are pursuing a policy of watchful waiting.

Under the circumstances the Editorial Committee for the revision of Wiley's "Principles and Practice of Agricultural Analysis" recommends that the publication of Vol. III be still further deferred until we can evaluate better the reception which awaits Volume II and until the economic conditions are more favorable for the bringing out of such a work.

C. A. BROWNE, *Chairman*

Approved.

REPORT OF COMMITTEE ON QUARTZ PLATE STANDARDIZATION AND NORMAL WEIGHT

Because of conditions beyond its control your committee has been unable to make a report on this subject for several years. The present explanatory statement regarding the existing situation is presented by the Chairman in lieu of a regular report and definite recommendations which the Committee expects to be able to make in 1933. Your Committee has been in the unfortunate position for a number of years of being unable to make definite recommendations because of international disagreements. It is of course obvious that the adoption by the Association of a definite basis for quartz plate standardization will affect the actual daily standardization of all saccharimeters. Your Committee has received numerous inquiries in the course of the past few years regarding the date on which these questions would be settled. Some years ago an error was discovered at the Bureau of Standards in the 100° point of the saccharimeter, but it was found impossible to secure international agreement for its correction. In recent years the situation has been further complicated by the abnormal increase which has been taking place in the use of the saccharimeter in every conceivable type of laboratory. It has found increased usage especially in research and commercial laboratories for uses other than the testing of carbohydrates. Thus the saccharimeter has become probably the most widely used optical instrument today for analytical work and measurement. Fortunately we have been able to reconvene the International Sugar Commission. It met in Amsterdam on the 5th of September, 1932, and discussed thirteen major subjects on analytical procedure. Most of these subjects are involved in the work of the Association in some form or other and specific recommendations were adopted by the Commission, not only on the 100° point of the saccharimeter and quartz plate standardization, but also on various analytical procedures. It is now believed that the way will be open during the next few months for your committee to make definite recommendations at the 1933 meeting. Your committee feels that the need for these recommendations has become especially acute in recent years and that the adoption of a definite procedure regarding them by the Association will greatly strengthen the Association's *Book of Methods.*

FREDERICK BATES
C. A. BROWNE
F. W. ZERBAN

Approved.

REPORT OF COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATIONS OF RESULTS ON FERTILIZERS AND LIMING MATERIALS

Final Adoption as Official

INTERPRETATION OF THE WORD "LIME" AS APPLIED TO FERTILIZERS

The term "lime" shall not be used in the registration, labeling, or guaranteeing of fertilizers or fertilizer materials unless the lime is in a form to neutralize soil acidity (the oxide, hydroxide or carbonate, or equivalent magnesia compounds).

Final Recommendation for Official Adoption

1. NET WEIGHTS

The weights appearing on packages of fertilizer, agricultural lime, and liming material shall always mean *net weights*.

2. CITRATE-SOLUBLE ("REVERTED") PHOSPHORIC ACID

Citrate-soluble ("reverted") phosphoric acid is that part of the total phosphoric acid in a fertilizer that is insoluble in water but soluble in a solution of citrate of ammonia according to the method adopted by the A.O.A.C.

3. AGRICULTURAL LIMING MATERIAL

Agricultural liming material is any substance that contains calcium and magnesium in condition and quantity suitable for use in neutralizing soil acidity.

Second Reading as Tentative

1. PHOSPHATE ROCK

Phosphate rock is a natural rock containing one or more calcium phosphate minerals of such purity and in sufficient quantity as to permit their use, either directly or after concentration, in the manufacture of commercial products.

2. SOFT PHOSPHATE WITH COLLOIDAL CLAY

Soft phosphate with colloidal clay is a very finely divided low-analysis by-product resulting from mining Florida rock phosphate by a hydraulic process, whereby the colloidal material settles most abundantly at points in artificial ponds and basins farthest from the washer, where it is later recovered upon the natural evaporation of the water.

3. PRECIPITATED BONE PHOSPHATE

Precipitated bone phosphate is a by-product from the manufacture of glue from bones and is obtained by neutralizing the hydrochloric acid solution of processed bone with calcium hydroxide. The phosphoric acid is largely present as dicalcium phosphate.

4. PRECIPITATED PHOSPHATE

Precipitated phosphate is a product consisting mainly of dicalcium phosphate obtained by neutralizing the acid solution of either phosphate rock or processed bone with calcium hydroxide.

5. HIGH CALCIC PRODUCTS

High calcic products are materials consisting of calcium and magnesium in a ratio of not less than nine parts of calcium to one part of magnesium.

6. HIGH MAGNESIC PRODUCTS

High magnesic products are materials consisting of calcium and magnesium in a ratio of more than one part of magnesium to nine parts of calcium.

7. BASIC LIME PHOSPHATE (BASED LIME PHOSPHATE)

"Basic" lime phosphate (based lime phosphate) is a superphosphate to which liming materials in calcium carbonate equivalents have been added in a quantity at least eight per cent (8%) in excess of that required to convert all water-soluble phosphate to the citrate-soluble form.

First Reading as Tentative

LIME

The word *lime* when applied to liming materials means calcium and magnesium oxides.

MONO-AMMONIUM PHOSPHATE (FERTILIZER GRADE)

Mono-ammonium phosphate (fertilizer grade) is a commercial salt made by combining phosphoric acid with ammonia. The commercial salt contains about ten per cent (10%) nitrogen and about forty-eight per cent (48%) of available phosphoric acid.

PHOSPHORIC ACID

The term *phosphoric acid* is used to designate the phosphoric oxide (P_2O_5).

POTASH

The term *potash* is used to designate the potassium oxide (K_2O).

UNIFORMITY IN USE OF TERMS "PHOSPHORIC ACID" AND "POTASH"

As the terms *phosphoric acid* and *potash* are universally used in the guarantee and analysis of fertilizers it is recommended that the same terms be used in reporting and discussing the results of analysis of those related materials.

For Future Consideration

Revision of official definitions of: Air-slaked lime, ground limestone, ground shell lime, and marl, ground shell marl.

J. W. Kellogg, *Acting Chairman*   W. H. MacIntire
R. N. Brackett   C. H. Jones
G. S. Fraps   H. D. Haskins, *Chairman*

Approved.

REPORT OF COMMITTEE ON RECOMMENDATIONS OF REFEREES

It is gratifying to report that the work done this year is of the usual high order of excellence and includes many reports that are exhaustive in character and of timely and practical interest. The committee is pleased to note a considerable increase in the number of papers submitted well in advance of the opening of the meeting. This has materially lightened the pressure of work that falls on the committee and has made it possible for its members to give more deliberate study to the reports and recommendations presented. Several items have been deleted from our schedule of subjects for study because there appears to be no urgent

need for work on them at this time; on the other hand, subjects of more immediate interest and greater promise of usefulness have been added. The subject of biological methods has recently been added to our schedule and material progress has been made in this direction. The committee in an earlier report has called attention to the desirability for the study of microchemical methods; and there is equally urgent need for the study of microbiological methods. Both of these subjects are now being added to our schedule of topics for active study.

E. M. BAILEY, *Chairman*

Approved.

REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES

By R. N. BRACKETT (Clemson College, S. C.), *Chairman;*
H. H. HANSON and H. R. KRAYBILL

INSECTICIDES AND FUNGICIDES

It is recommended that Method II for the determination of lead oxide and copper in Bordeaux-lead arsenate mixtures[1] be adopted as official (first action) and that it be further studied.

Approved.

FLUORINE COMPOUNDS

It is recommended—

(1) That the Willard-Winter[2] method for the determination of fluorine be studied further.

Approved.

(2) That collaborative and experimental study of that method and also of the present tentative method for the determination of fluorine (*Methods of Analysis, A.O.A.C.,* 1930, 59), be conducted.

Approved.

CAUSTIC POISONS

It is recommended that the position of Referee on Caustic Poisons be discontinued and that the subject of caustic poisons be given to the Referee on Insecticides and Fungicides.

Approved.

SOILS AND LIMING MATERIALS

It is recommended that the wet combustion of soils be studied with the object of formulating an acceptable method.

Approved.

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[1] *This Journal,* 5, 289 (1932).
[2] *Ind. Eng. Chem. Anal. Ed.,* 5, 7 (1933).

REACTION VALUE OF SOILS

Alkaline Soils

It is recommended that the procedure for the determination of the *p*H value of soils be considered further.

Approved.

Acid Soils

The associate referee reviewed the status of knowledge regarding soil acidity but recommends no specific methods at this time.

The committee approves the implied recommendation of the associate referee that study of this subject be continued.

Approved.

LESS COMMON METALS IN SOILS

The associate referee recommended that the combustion method for the determination of iodine in soils[1] be made tentative and be subjected to cooperative study next year and that the fusion method[1] be made an alternative method under the same conditions.

The committee does not approve. It is recommended that after a collaborative study has been conducted next year the method be recommended for adoption as tentative or as official, as the experience may warrant.

Approved.

FEEDING STUFFS

It is recommended--

(1) That the quantitative determination of sodium chloride in feeding stuffs be studied further.

Approved.

(2) That the following recommendation, which was approved last year, be continued: That the methods of preparation of solution and determination of sugars in feeding stuffs (*Methods of Analysis, A.O.A.C.,* 1930, 281), adopted as official (first action) in 1930, be further studied.

STOCK FEED ADULTERATION

No report was submitted.

It is recommended that the following recommendation, approved last year, be continued: That methods given in the 1930 report of the associate referee for the detection of traces of potassium iodide, copper sulfate and ferrous sulfate[2] be adopted as tentative and further studied.

Approved.

MINERAL MIXED FEEDS

It is recommended—

(1) That the tentative method for the determination of lime in mineral feeds (*Methods of Analysis, A.O.A.C.,* 1930, 287) be further studied.

Approved.

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[1] *Ind. Eng. Chem., Anal. Ed.,* **4**, 214 (1932).
[2] *This Journal,* **14**, 143 (1931).

(2) That the work on the comparison of the Knapheide and Lamb method[1] with other methods for the determination of iodine be continued.

Approved.

(3) That methods for the determination of iodine in organic compounds be further studied.

Approved.

MOISTURE

No report was submitted.

It is recommended that the work be continued.

Approved.

SOLVENTS FOR DETERMINATION OF FAT IN FEEDING STUFFS

It is recommended that this work be continued another year.

Approved.

HYDROCYANIC ACID IN GLUCOSIDE-BEARING MATERIALS

It is recommended that the subject of hydrocyanic acid in glucoside-bearing materials be further studied, and that the tentative methods for this determination (*Methods of Analysis, A.O.A.C.*, 1930, 287) be subjected to collaborative study.

Approved.

BIOLOGICAL METHODS FOR THE DETERMINATION OF COD-LIVER OIL IN FEED MIXTURES

It is recommended—

(1) That the method for vitamin D assay incorporated in the report of the associate referee be modified and collaboratively studied.

Approved.

(2) That any other methods available for the detection of cod-liver oil in mixed feeds be studied.

Approved.

SUGARS AND SUGAR PRODUCTS

It is recommended—

(1) That the changes and additions suggested by the associate referee for elucidating the description for the preparation and use of clarifying reagents in Sections 18a, b, d, e, pp. 368–9, *Methods of Analysis, A.O.A.C.*, 1930, be adopted (see p. 78).

Approved for first action on a change in an official method.

(2) That a change in description of Section 22 (a), p. 371, *Methods of Analysis, A.O.A.C.*, 1930, be made (see p. 79).

Approved for first action on a change in an official method.

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[1] *J. Am. Chem. Soc.*, **50**, 2121 (1928).

HONEY

It is recommended—

(1) That studies on methods for determining the diastatic activity of honey be continued.

Approved.

(2) That the subject of the determination of moisture in honey be given critical attention.

Approved.

MAPLE PRODUCTS

It is recommended—

(1) That the changes suggested by the associate referee in the wording of the tentative method for preparation of samples of maple sirup, *Methods of Analysis, A.O.A.C.*, 1930, 103 (a), 2, p. 391, be made (see p. 79).

Approved.

(2) That the changes suggested by the associate referee in the wording of the tentative method for preparation of sample of maple sugar and other solid or semi-solid maple products, *Methods of Analysis, A.O.A.C.*, 1930, 103 (b), 2, p. 391, be made (see p. 79).

Approved.

(3) That the additions suggested by the associate referee be made to the official method for the determination of moisture in maple products, *Methods of Analysis, A.O.A.C.*, 1930, 104 (a), (see p. 79).

Approved for change in an official method (first action).

(4) That the advisability of recognizing the densimetric methods of determining solids (*Methods of Analysis, A.O.A.C.*, 1930, 5 and 6, p. 364) as applicable to maple sirup receive consideration.

Approved.

(5) That consideration be given to the alteration of the directions for polarization suggested by the associate referee so as to allow the use of alumina cream and of normal lead acetate as clarifying agents.

Approved.

(6) That in 107, *Methods of Analysis, A.O.A.C.*, 1930, p. 392, the caption *Sucrose in the Absence of Raffinose* be deleted and the directions altered to read: "Calculate from the results of 105, using the appropriate formula from 22 or 23."

Approved for change in an official method (first action).

(7) That in the directions for Winton lead value (tentative), those for preparation of the reagent, 115, be changed as suggested by the associate referee (see p. 80).

Approved.

(8) That in the directions for "Canadian lead number (Fowler modification) tentative," those for preparation of the reagent, **118,** be changed as suggested by the associate referee (see p. 80).
Approved.

(9) That experimentation on lead values be continued and the effect of varying the proportions of litharge and normal lead acetate be studied.
Approved.

(10) That in reference to the Canadian lead value the effect of error in measuring the volume of the reagent be studied.
Approved.

(11) That sections **120** and **121** be revised as suggested by the associate referee and remain as official (first action), (see p. 80).

DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS

It is recommended—

(1) That the official refractometric method, section **7,** be amended as suggested by the associate referee (see p. 81).
Approved.

(2) That further study be given density methods applicable to molasses, sirup, and other materials containing large quantities of non-sugar solids.
Approved.

(3) That the committee appointed to study the question of a standard scale for immersion be continued.
Approved.

POLARISCOPIC METHODS

It is recommended that study be continued along the lines covered by the recommendations made and approved last year.[1]
Approved.

CHEMICAL METHODS FOR REDUCING SUGARS

No report was submitted.

It is recommended that recommendations Nos. 1 and 3, made last year on this subject,[1] be repeated.
Approved.

FERTILIZERS
PHOSPHORIC ACID

It is recommended—

(1) That the first of the alternative methods for preparing ammonium citrate solution, *Methods of Analysis, A.O.A.C.,* 1930, p. 17, section **13**

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[1] *This Journal,* **14, 43** (1932).

(1), be deleted (first action). (This involves also deletion of the preceding sentence "Prepare according to either, etc. . . ."

Approved.

(2) That the wording of the second method for the preparation of ammonium citrate solution be changed as suggested by the associate referee (see p. 68).

Approved.

(3) That the study of the method for the determination of citrate-insoluble phosphoric acid be continued.

Approved.

(4) That a collaborative study be made of methods of determining free acid in superphosphates.

Approved.

NITROGEN

It is recommended—

(1) That the study of any new processes which may lead to a more accurate determination of ammoniacal nitrogen in the presence of urea and cyanamide be continued.

Approved.

(2) That a comparative study be made of the catalysts selenium and mercury in the determination of total nitrogen in fertilizer materials and mixed fertilizers.

Approved.

HIGH ANALYSIS FERTILIZERS

It is recommended—

(1) That the determination of moisture in hygroscopic materials, especially mixtures of calcium and ammonium nitrates, be studied further.

Approved.

(2) That the uniformity of the parcels comprising fertilizer shipments be studied collaboratively; that a careful review of the work already done by the association on this subject be made before undertaking any collaborative studies; and that the associate referee confer with the Committee on Sampling.

Approved.

(3) That to obtain the consensus of opinion regarding the degree of accuracy practicable for the analysis of shipments of high-analysis fertilizers, the question be referred to the Committee on Definitions of Terms and Interpretation of Results on Fertilizers.

Approved.

POTASH

It is recommended—

(1) That further time be given to try out suggested modifications of the official method for the determination of potash in order that some of these may be used in collaborative study.

Approved.

(2) That the method for the determination of chlorine in fertilizers (*Methods of Analysis, A.O.A.C.*, 1930, 30), (official, first action, 1928), be adopted as official, final action.

Approved.

PLANTS

It is recommended—

(1) That the method for the determination of fluorine referred to in the report of the Referee on Plants be further studied with the purpose of making it applicable to plant materials and if feasible that collaborative work be done.

Approved.

(2) That an associate referee be appointed to study methods for the determination of sodium in plants.

Approved.

(3) That in *Methods of Analysis, A.O.A.C.*, 1930, p. 102, par. 5 and footnote, "10–50 g." be adopted as official, final action.

Approved.

(4) That the method for the determination of ferric and aluminum oxides, now marked official, and the expression in parentheses "applicable to plant materials other than seeds" (*Methods of Analysis, A.O.A.C.*, 1930, p. 103, preceding par. 6) be deleted (final action).

Approved.

(5) That the methods for the determination of manganese, calcium, and magnesium, now marked official, and the expression in parentheses "applicable to plant materials other than seeds" (*Methods of Analysis, A.O.A.C.*, 1930, p. 104, preceding par. 9) be deleted (final action).

Approved.

(6) That the method for the determination of calcium, official, first action (*Methods of Analysis, A.O.A.C.*, 1930, p. 104, par. 9), be adopted as official (final action).

Approved.

(7) That the micro method for the determination of calcium, now tentative (*Methods of Analysis, A.O.A.C.*, 1930, p. 105, pars. 10–12) be adopted as official (first action).

Approved.

(8) That the method for the determination of magnesium, official, first action (*Methods of Analysis, A.O.A.C.*, 1930, p. 106, par. 13) be adopted as official (final action).

Approved.

(9) That the magnesium nitrate method for the determination of sulfur, official, first action (*Methods of Analysis, A.O.A.C.*, 1930, p. 110, pars. 26–27), be adopted as official (final action).

Approved.

(10) That Method I for the determination of phosphorus, official, first action (*Methods of Analysis, A.O.A.C.*, 1930, p. 110, par. 28) be adopted as official (final action).

Approved.

(11) That Method II, the micro method for the determination of phosphorus, now tentative (*Methods of Analysis, A.O.A.C*, 1930, p. 110, pars. 29–31) be adopted as official (first action).

Approved.

PREPARATION OF PLANT MATERIAL FOR ANALYSIS

It is recommended—

(1) That the method of preparation of plant materials for analysis, adopted tentatively in 1929 and as official (first action) in 1931, be adopted as official (final action).

Approved.

(2) That study of this subject be discontinued.

Approved.

LESS COMMON METALS

It is recommended that the method for the spectroscopic determination of boron be studied collaboratively with a view to recommending its adoption as tentative or official (first action) next year, as experience warrants.

TOTAL CHLORINE

No formal report was submitted.

It is recommended that this work be continued.

Approved.

CARBOHYDRATES IN PLANTS

It is recommended—

(1) That the use of invertase in the determination of sucrose be studied further.

Approved.

(2) That further studies be made upon methods for the determination of reduced copper.

Approved.

(3) That studies be made upon the determination of starch.

Approved.

Recommendations 1, 2 and 3[1] were approved last year as official (first action). The associate referee is not ready to recommend final action at this time.

FORMS OF NITROGEN

It is recommended—

(1) That collaborative study of the method suggested by the associate referee for the determination of nitrate nitrogen be undertaken.

Approved.

(2) That the recommendation of the associate referee in 1931 (through error not recorded in Committee A report of 1931) for the adoption of methods cited in the report for determinations of ammonia and "free nicotine" in tobacco[2] as tentative be adopted.

LIGNIN

It is recommended that further work be done on simplifying the method for the determination of lignin.

Approved.

ENZYMES

It is recommended that the method for the determination of catalase submitted by the referee in a contributed paper[3] be subjected to trial and criticism in various laboratories of the Association which are interested in the subject, with the object of ascertaining (1) how satisfactory the method is in the hands of other workers; (2) any modifications or improvement which might facilitate the analysis or considerably increase the accuracy of the determination; and (3) the range of agricultural products with which satisfactory results may be obtained.

Approved.

PAINTS, PAINT MATERIALS, AND VARNISHES

No report was submitted.

It is recommended that Recommendation 2[4] made last year be repeated.

Approved.

[1] *This Journal*, 15, 46 (1932).
[2] *Ibid.*, 14, 228 (1932).
[3] *Ibid.*, 15, 486 (1932).
[4] *Ibid.*, 47.

REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES

By L. E. WARREN (U. S. Food and Drug Administration, Washington, D. C.), *Acting Chairman;* H. C. LYTHGOE and A. G. MURRAY

NAVAL STORES

No work was done on spirits of turpentine. Some experiments were started on the analysis of rosin.

It is recommended that the topic be continued with special reference to methods for the analysis of rosin.

Approved.

BEERS, WINES AND DISTILLED LIQUORS

No work was reported.

It is recommended that the topic be continued.

Approved.

SPECIFIC GRAVITY AND ALCOHOL

It is recommended that the subject be closed.

Approved.

DRUGS

CRUDE DRUGS

The associate referee reported progress on the study of aconite.

It is recommended that the subject be continued.

Approved.

RADIOACTIVITY IN FOODS AND DRUGS

Advances are being made in this highly technical subject.

It is recommended that the topic be continued.

Approved.

EMODIN-BEARING DRUGS

This subject has been studied for a number of years. Two methods have now been formulated, one for cascara preparations and another for aloin. Satisfactory results appear to have been obtained from trials of each.

It is recommended—

(1) That the method for the determination of cascara preparations submitted by the associate referee be adopted as tentative (see p. 81).

Approved.

(2) That the method for the determination of aloin submitted by the associate referee[1] last year be adopted as tentative.

Approved.

(3) That the subject be closed for the present.

Approved.

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[1] *This Journal,* 15, 407 (1932).

MERCURIALS

No collaborative work was done this year.

It is recommended that the subject be continued to include mercury-containing dyes represented as antiseptics.

Approved.

MICROCHEMICAL METHODS FOR ALKALOIDS

Since 1926 microchemical methods for the identification of alkaloids have been studied. Of twenty-one alkaloids studied satisfactory methods for the identification of nineteen have been found. All but two of these have been adopted by the association. This year methods for nicotine and sparteine were studied.

It is recommended—

(1) That the tests and descriptions submitted by the associate referee for nicotine be adopted as tentative (see p. 82).

Approved.

(2) That the tests and descriptions submitted by the associate referee for sparteine be adopted as tentative (see p. 82).

Approved.

HYPOPHOSPHITES

Some progress was made this year.

It is recommended that the work be continued.

Approved.

SANTONIN

Considerable progress in this difficult subject was reported.

It is recommended that the topic be continued with the suggestion that the Claus method[1] be subjected to collaborative study.

Approved.

ETHER

It is recommended that the subject be continued with special reference to the influence of essential oils or other volatile substances on the determination of ether.

Approved.

BENZYL COMPOUNDS

This is a new topic. A method for the assay of benzyl alcohol has been devised and it gives promising results.

It is recommended that the subject be continued.

Approved.

ALCOHOL IN DRUGS

No report was submitted.

It is recommended that the subject be closed.

Approved.

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[1] *Pharm. Weekblad,* **68,** 414 (1931); *J. Pharm. Belg.,* **13,** 427 (1931); *C. A.,* **25,** 3432 (1931).

SMALL QUANTITIES OF IODIDES IN MIXTURES

It is recommended that the subject be closed.
Approved.

PHENOLSULFONATES

A method worked out by the associate referee appears to give satisfactory results.

It is recommended—

(1) That the method reported by the associate referee be adopted as tentative see p. 83).
Approved.

(2) That the subject be closed.
Approved.

SULFONAL AND TRIONAL

This subject has been studied for several years. The associate referee studied two methods, and these have been combined into one by the Referee on Drugs.

It is recommended—

(1) That the method be adopted as tentative and retained in that status (see p. 83).
Approved.

(2) That the subject be closed.
Approved.

GUAIACOL

It is recommended that the subject be continued.
Approved.

BROMIDE-BROMATE METHODS

No collaborative work has been done on this subject. Although it is relatively unimportant, it is recommended that the subject be continued.
Approved.

IPOMEA AND JALAP

A method has been worked out for the assay of these drugs; it is identical for each and gives satisfactory results.

It is recommended—

(1) That the method submitted by the associate referee be adopted as tentative (see p. 84).
Approved.

(2) That the subject be closed.
Approved.

PODOPHYLLUM

It is recommended that the subject be included in the new topic "Resins and Oleoresins."

Approved.

RHUBARB AND RHAPONTICUM

It is recommended that the topic be continued.

Approved.

CALCIUM GLUCONATE

Last year a method was worked out for the assay of the gluconate ion. The method was based upon the change in optical rotation due to the addition of uranium salts. The polarimetric method appears promising.

It is recommended that the subject be continued.

Approved.

TETRACHLORETHYLENE

Some progress was reported.

It is recommended that the subject be continued.

Approved.

HEXYLRESORCINOL

A new modification of the Koppeschaar procedure[1] to effect complete bromination of the hexylresorcinol by raising the initial temperature and lengthening the time for reaction was studied. The results were not so uniform as was desired. Therefore—

It is recommended that the subject be continued with a view to obtaining more nearly uniform results and discovering more suitable qualitative tests.

Approved.

ERGOT AKALOIDS

This is a new topic. One method has been studied in a collaborative way with good results. Because only a limited number of collaborators participated in the work, and in view of the importance of the topic—

It is recommended that the subject be continued with the suggestion that studies be made of the use of artificial light, hydrogen peroxide, and other oxidizing agents for the development of colors, and of the substitution of sulfuric acid by phosphoric acid for the production of purer tints.

Approved.

MICROCHEMICAL METHODS FOR SYNTHETICS

This is a new topic. Chinosol, benzocaine, pyridium and cinchophen were studied this year.

It is recommended—

(1) That the methods reported by the associate referee for chinosol, benzocaine, pyridium and cinchophen be adopted as tentative (see p. 84).

Approved.

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[1] *Z. anal. Chem.*, 15, 233 (1876).

(2) That the topic be continued with a view to studying new items.
Approved.

A slight change in the method of determining free salicylic acid in acetylsalicylic acid was suggested by the referee.

It is recommended that the change recommended by the referee be adopted.
Approved.

It is recommended that the topic be continued.
Approved.

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES

By J. O. CLARKE (U. S. Food and Drug Administration, Chicago, Ill.), *Chairman;* G. G. FRARY and H. A. LEPPER

It is recommended—

(1) That the tentative method for the determination of phosphoric-pentoxide (*Methods of Analysis, A.O.A.C.*, 1930, 248), as revised by the associate referee, be adopted as official (first action); that the heading of the method be changed from "Phosphoric-Pentoxide" to "Total Phosphoric acid (P_2O_5)"; and that "(b) Olive Oil" under "Reagents" and the sentence "Add to the whites 0.5 cc. of olive oil" under "Preparation of Solution" be deleted.
Approved.

(2) That the method for the determination of fat by acid hydrolysis,[1] as revised by the associate referee, be adopted as tentative and further studied collaboratively (see p. 73).
Approved.

(3) That the extraction modification of the method for the determination of lipoids and lipoid phosphoric acid (P_2O_5) described in the report of the associate referee be adopted as tentative and further studied collaboratively after the following change has been made: Delete the section "and pour as rapidly as possible onto an $18\frac{1}{2}$ cm. folded filter, covering the funnel with a watch-glass during the filtration, and collecting the filtrate in a 200 cc. Erlenmeyer flask" and substitute therefore the following clause, "allow to stand until clear, and transfer a 50 cc. aliquot to a 250 cc. beaker." (See p. 73.) It is also suggested that a study be made

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[1] *This Journal,* 15, 313 (1932).

of the effect of insoluble solids noted by the general referee, with the object of establishing the definite corrections necessary.

Approved.

(4) That the method for the determination of reducing sugars and sucrose given in the associate referee's report be adopted as official (first action). It is also suggested that a study be made of the effect of insoluble solids noted by the general referee, with the object of establishing the definite corrections necessary.

Approved.

(5) That that part of the tentative method for the determination of water-soluble nitrogen and water-soluble nitrogen precipitable by 40 per cent alcohol (crude albumin nitrogen)[1] applicable to liquid eggs be adopted as official (first action) and that the part relating to dried eggs be further studied. It is also recommended that this method and others for the determination of water-soluble nitrogen precipitable by 40 per cent alcohol be designated as "crude albumin nitrogen."

Approved.

(6) That the tentative method for the determination of chlorine (*Methods of Analysis, A.O.A.C.*, 1930, 249) be adopted as official (first action) after the reagent "(b) Olive Oil" has been deleted.

Approved.

(7) That the study of the methods for unsaponifiable matter and its constituents be continued.

Approved.

(8) That work to perfect the method for acid-soluble phosphoric acid (P_2O_5) be continued.

Approved.

(9) That the qualitative test for glycerol described in the 1931 report of the referee[2] be amended by changing the last sentence to read—"In the presence of glycerol (due to acrolein) a strong pink color develops within 1 minute and becomes a deep violet-red within 5 minutes," that it be adopted as tentative, and that it be further studied with a view to adoption as official.

Approved.

(10) That the quantitative method for glycerol described in the 1931 report of the referee[3] be adopted as tentative, with the heading "Glycerol (not applicable in presence of added sugars)"; that further studies be made to determine the cause of the high blank; and that further studies

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[1] *This Journal*, 15, 75 (1932).
[2] *Ibid.*, 331.
[3] *Ibid.*, 334.

on the precipitation method described in this report be made, to be followed by collaborative work.

Approved.

(11) That methods for determining ammonia nitrogen as an index of decomposition in liquid egg be studied.

Approved.

(12) That the rapid method for the determination of acidity of ether extract described in the associate referee's report for 1931[1] be studied further with a view to making it applicable to both liquid and dried eggs.

Approved.

(13) That since a new basis of greater accuracy of calculating egg solids has resulted from recent work,[2] sections 23 and 24, (*Methods of Analysis, A.O.A.C.*, 1930, p. 249) be deleted.

Approved.

(14) That sections 16, 17, 18, 19 and what should be designated as 20 and 21, and section 22, pp. 248 and 249, be designated as 14, 15, 16, 17, 18, 19 and 20, respectively, and be followed by the present sections 14 and 15 on methods for detection of decomposition as sections 21 and 22, respectively.

Approved.

FOOD PRESERVATIVES

It is recommended—

(1) That in the first line on p. 345 (*Methods of Analysis, A.O.A.C.*, 1930), after the word "hour" there be inserted "(1½ hours in case of dried fruits)."

Approved.

(2) That the Monier-Williams method be designated as "applicable in the presence of other volatile sulfur compounds" and be placed following the official distillation method, section 31, p. 343, under the heading "Total Sulfurous Acid."

Approved.

(3) That collaborative work be carried out with the Monier-Williams method with a view to its official adoption and recommendations for the limitation of the number of methods for the determination of sulfur dioxide be considered.

Approved.

(4) That in the method for boric acid, p. 340, section 16, the directions be changed to provide for the use of 1–2 grams of mannitol instead of 10 grams for the titration. (This is in keeping with recognized practices and with directions for boric acid in fertilizers.)

Approved.

[1] *This Journal*, **15**, 341 (1932).
[2] *Ibid.*, 310.

(5) That study of methods for the determination of saccharine be continued.

Approved.

(6) That a change of 10% to 1% solution $CuSO_4$ in line 4 of section 3, p. 335, made official, first action, 1930,[1] be made official, final action.

Approved.

COLORING MATTERS IN FOODS

It is recommended—

(1) That additional collaborative work be devoted to the separation and identification of coloring matters in alimentary pastes.

Approved.

(2) That the study of the quantitative separation and estimation of the recently permitted dyes be continued.

Approved.

(3) That additional investigation be carried out on the qualitative separation and identification of light green S F yellowish, brilliant blue F C F, and fast green F C F.

Approved.

METALS IN FOODS

It is recommended—

(1) That the bromate method submitted in 1930 and modified in 1931 by the associate referee be adopted as tentative for the determination of arsenic on fresh fruits, and that it be further studied, with emphasis given to the influence of interfering substances. (See p. 75.)

Approved.

(2) That the arsine distillation method for the determination of arsenic be studied.

Approved.

(3) That the Gutzeit method be studied from points of view of technic and interfering substances.

Approved.

(4) That methods for the determination of copper and zinc be further studied.

Approved.

(5) That studies on methods for the determination of tin be discontinued for the present.

Approved.

(6) That methods for the determination of lead be further studied.

Approved.

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[1] *This Journal*, 14, 76 (1931).

(7) That methods for the determination of fluorine be studied.

Approved.

FRUITS AND FRUIT PRODUCTS

It is recommended—

(1) That study of a refractometric method for the determination of soluble solids in fruit products be continued.

Approved.

(2) That the study of the analysis of fruit products by a method of adjusted hydrogen-ion concentration be continued.

Approved.

(3) That study of methods for the determination of iron and aluminum in fruit ash be continued.

Approved.

(4) That study of the changes in the character of sugar when dried in the presence of acids be continued.

Approved.

(5) That study of methods for the determination of fruit acids be continued.

Approved.

(6) That methods for the determination of moisture in dried fruit be studied.

Approved.

(7) That the word "official" be deleted from section **49**, p. 276, *Methods of Analysis, A.O.A.C.*, 1930.

The Monier-Williams method for sulfurous acid, p. 344, recognized as applicable to dried fruits after slight modification, recommended this year, is tentative only. The wording of section **49** is a general reference only. The designation of the status of the methods should be given in the chapter in which the methods are detailed to prevent any misunderstanding.

Approved.

CANNED FOODS

It is recommended—

(1) That methods for determining quality factors and fill of container in canned foods be studied.

Approved.

(2) That methods for solids in tomato products be studied.

Approved.

DAIRY PRODUCTS
MILK

It is recommended that the General Referee on Milk direct such studies as he deems advisable.

Approved.

MALTED MILK

It is recommended—

(1) That the determination of the Reichert-Meissl number of the fat of malted milk by the use of the method of extraction reported by the associate referee in 1931[1] be studied collaboratively and that the determination of the total amount of butter fat in malted milk be also studied.

Approved.

(2) That a method for the determination of citric acid in malted milk be studied.

Approved.

(3) That a method for the determination of casein in malted milk be studied.

Approved.

DRIED MILK

It is recommended—

(1) That study be made of the sampling of dried milk.

Approved.

(2) That further study be made of the distillation method for determining moisture in dried milk.[2]

Approved.

BUTTER

It is recommended—

(1) That the subject of sampling tub, cube and print butter be thrown open for further study.

Approved.

(2) That the associate referee study the composition of the individual units in the churning with a view to recommending a suitable sampling procedure to cover the unit package as well as the average unit in the churning or batch.

Approved.

(3) That the modified stirrer method outlined by the associate referee receive further collaborative study.

Approved.

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[1] *This Journal*, 15, 529 (1932).
[2] *Ibid.*, 8, 295 (1925); 15, 527 (1932).

(4) That the methods for determining salt and curd (*Methods of Analysis, A.O.A.C.*, 1930, 237) receive further study by the associate referee, especially with a view to determining the best method of removing the last traces of fat.

Approved.

(5) That the associate referee inaugurate some work for the purpose of developing methods for distinguishing sweet cream butter from neutralized butter, with special reference to detection of neutralizers and starters, but that these studies have no precedence over the others recommended.

Approved.

(6) That further collaborative study be given to the alundum Gooch crucible method (*Methods of Analysis, A.O.A.C.*, 1930, 236, 77), for the determination of fat in butter.

Approved.

<div align="center">CHEESE</div>

(1) That the method developed by Huebner for determining lactose and sucrose in cheese,[1] as modified by the associate referee, be adopted as a tentative method.

Approved.

(2) That further work be done on the detection and estimation of added preservatives or other ingredients, such as gums, etc., in process cheese and that the work on gums be carried out in collaboration with the Referee on Gums in Foods.

Approved.

(3) That further work on the P_2O_5–CaO ratio be continued.

Approved.

(4) That the associate referee present recommendations on citric and tartaric acid, now official (first action), for final action if justified.

Approved.

<div align="center">ICE CREAM</div>

It is recommended that the Nebraska[2] and Illinois[3] methods for the determination of fat in ice cream by the modified Babcock procedure be studied collaboratively.

Approved.

<div align="center">MILK PROTEINS</div>

It is recommended—

(1) That Method II, Albumin, section 11, p. 216, *Methods of Analysis,*

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[1] *This Journal*, 13, 243 (1930).
[2] Univ. of Nebraska Agr. Expt. Sta. Bull. 246.
[3] Univ. of Illinois Agr. Expt. Sta. Bull. 360.

A.O.A.C., 1930, be deleted as official (final action). First action was taken in 1930.[1]

Approved.

(2) That Method II—Tentative, for the determination of casein (*Methods of Analysis, A.O.A.C.*, 1930, 215), be continued as a tentative method and further studied.

Approved.

(3) That the revised method proposed by the associate referee for the determination of albumin be further studied.

Approved.

(4) That a method for the determination of casein in milk powders be studied and that the work be carried out in collaboration with the Associate Referee on Dried Milk.

CEREAL FOODS

Flour, Alimentary Pastes and Baked Products

It is recommended—

(1) That special (non-collaborative) studies be conducted on the methods for the determination of unsaponifiable matter in the fat of flour, alimentary pastes and baked products, in conjunction with the same study on eggs.

Approved.

(2) That further study be made of the tentative method for the determination of water-soluble protein nitrogen precipitable by 40 per cent alcohol in flour, alimentary pastes and baked products, in conjunction with similar methods for eggs. It is also recommended that this method be designated "Crude Albumin Nitrogen."

Approved.

(3) That comparative tests be made of foreign and domestic methods of chemical analysis used as measures of evaluating flour, alimentary pastes and baked products.

Approved.

(4) That the colorimetric method proposed by the associate referee for the determination of the hydrogen-ion concentration in flour be made tentative (see p. 72). That further study be also made to determine what color standard is best adapted for such pH work. That the applicability of the color method to alimentary pastes and baked products be studied.

Approved.

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[1] *This Journal*, 14, 57 (1931).

(5) That further study be made of the method of ashing flour, alimentary pastes and baked products with the object of reducing the time of combustion.

Approved.

(6) That the acid hydrolysis method for the determination of fat in alimentary pastes and baked products be further studied.

Approved.

(7) That the method modified by the associate referee for the determination of phosphorus in eggs be studied collaboratively regarding its applicability to flour, alimentary pastes and baked products.

Approved.

(8) That the method as modified by the associate referee for the determination of lipoids and lipoid-phosphorus in flour, alimentary pastes and baked products be further studied.

Approved.

(9) That the modification of the tentative quantitative method (*Methods of Analysis, A.O.A.C.*, 1930, 173, 39) proposed by the associate referee for the determination of chlorine in bleached flour be further studied; that collaborative work be conducted on the method for the detection of benzoyl peroxide (as benzoic acid) in flour.

Approved.

(10) That further collaborative studies be made of the method as modified by the associate referee for the determination of diastatic activity in flour.

Approved.

(11) That the study of methods for the determination of color in flour be resumed, special attention being paid to the use of the Munsell or similar apparatus.

Approved.

(12) That the Rask method for starch determination in flour, as modified by Munsey and reported in the associate referee's report, be further studied collaboratively and that further work on the diastase-acid hydrolysis method be discontinued.

Approved.

(13) That the tests suggested by the associate referee for the detection of rye flour in wheat flour and bread be studied collaboratively.

Approved.

(14) That methods for the determination of CO_2 in self-rising flour be further studied.

Approved.

(15) That the vacuum method (official, first action) for the determination of moisture in air-dried bread be made official (final action).
Approved.

(16) That the method for determining moisture in bread by heating at 130° for one hour (official, first action, *Methods of Analysis*, A.O.A.C., 1930, 177, 49) be made official (final action).
Approved.

(17) That both methods referred to in Recommendations 15 and 16 be made official (first action) for the determination of moisture in air-dried baked products other than bread.
Approved.

(18) That the method for the determination of crude fiber in bread and alimentary pastes (official, first action) be made official (final action); that the method be made tentative for baked products other than bread and studied collaboratively.
Approved.

(19) That study be made of methods to determine milk solids in bread.
Approved.

(20) That the method for the determination of chlorides in flour and baked products be further studied.
Approved.

(21) That the tentative method for making a standard baking test (*Methods of Analysis*, A.O.A.C., 1930, 178) be further studied.
Approved.

(22) That the tentative method for preparation of samples of alimentary pastes for analysis (*Methods of Analysis*, A.O.A.C., 1930, 180) be further studied.
Approved.

(23) That methods of determining chlorides in baked products (official, first action) be further studied before final action is taken.
Approved.

VINEGARS

It is recommended—
(1) That methods for the determination of total and soluble ash be further studied, with particular attention given to the use of sucrose or other substances for reducing the time of heating and to the temperature of ashing.
Approved.

(2) That the methods for the determination of phosphoric acid be further studied in connection with the studies on ash.
Approved.

(3) That the official method for the determination of total solids be studied, especially with reference to its application to vinegars high in solids, such as malt vinegar.

Approved.

(4) That the procedure using diphenylamine as an indicator for the titration of glycerol be studied collaboratively on samples of vinegar, in comparison with the present procedure.

Approved.

FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended—

(1) That Method I for the determination of essential oil in extracts and toilet preparations, given in the referee's report for 1931[1] and revised in the report this year, be made tentative for extracts of anise, lemon, nutmeg, orange, rosemary, thyme, wintergreen and methyl salicylate.

Approved.

(2) That Method II for the determination of essential oil in extracts and toilet preparations, given in the referee's report for 1931[1] and revised in the report this year, be made tentative for extracts of cinnamon and cloves.

Approved.

(3) That these methods be subjected to further collaborative work, including tests on extracts not included in the above recommendations.

Approved.

MEAT AND MEAT PRODUCTS

It is recommended—

(1) That the method for the determination of salt detailed by the referee (see p. 75) be adopted as tentative.

Approved.

(2) That methods for the determination of nitrates and nitrites be studied.

Approved.

SEPARATION OF MEAT PROTEINS

It is recommended that work on the separation of meat proteins be discontinued for the present.

Approved.

GELATIN

It is recommended—

(1) That the methods of preparation of the sample be made the subject of collaborative study during the coming year.

Approved.

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[1] *This Journal*, 15, 539 (1932).

(2) That the tentative method for the determination of arsenic (*Methods of Analysis*, A.O.A.C, 1930, 103) be made the subject of collaborative study. It is also suggested that the Referee on Gelatin confer with the Referee on Heavy Metals to correlate the procedures as much as possible.

Approved.

SPICES AND OTHER CONDIMENTS

It is recommended—

(1) That study be continued on the determination of volatile oils in spices.

Approved.

(2) That methods for the determination of starch and sugars in prepared mustard be studied.

Approved.

(3) That present tentative methods for total solids, *Methods of Analysis*, A.O.A.C., 1930, p. 355, sec. 36, total acid (sec. 40), oil (p. 356, sec. 41), lecithin P_2O_5 (section 43), and egg solids (section 44) be deleted.

Approved.

(4) That the methods proposed by the referee for the determination of total solids, total acidity, total nitrogen and total P_2O_5 be adopted as official (first action).

Approved.

(5) That the method proposed by the referee for the determination of fat be adopted as tentative.

Approved.

(6) That the procedure for calculation of composition proposed by the referee be adopted as tentative.

Approved.

CACAO PRODUCTS

It is recommended—

(1) That the method for the determination of crude fiber in milk chocolates[1] be adopted as official (final action).

Approved.

(2) That further work be done on the method for the determination of milk proteins in milk chocolate described in the report of the associate referee for 1932 on milk proteins in milk chocolate.

Approved.

(3) That the method for the quantitative determination of foreign fat

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[1] *This Journal*, 15, 72 (1932).

by means of the "A" and "B" numbers, described in the report of 1931 on cacao butter, be further studied.

Approved.

(4) That the methods for sucrose in chocolate and lactose in milk chocolate described in the report for 1931 on sucrose and lactose be adopted as tentative and studied further collaboratively with a view to their adoption as official.

Approved.

(5) That the present tentative method for sucrose and lactose in cacao products, *Methods of Analysis, A.O.A.C.*, 1930, 158, be dropped.

Approved.

COFFEE AND TEA

It is recommended—

(1) That the study of determining caffeine in so-called decaffeinated coffees be continued.

Approved.

(2) That directions in the method for the determination of caffeine in tea, section **42,** p. 155, *Methods of Analysis, A.O.A.C.*, 1930, making determination of nitrogen compulsory instead of optional, made official, first action, 1930,[1] be made official (final action).

Approved.

FATS AND OILS

It is recommended—

(1) That the vacuum oven method for the determination of moisture and volatile matter in fats and oils[2] be made official (final action).

Approved.

(2) That no further work be done on the Albert method for the determination of the acid and saponification values of dark-colored oils.

Approved.

(3) That methods for the analysis of cottonseed be studied.

Approved.

(4) That a study, including collaborative work, be undertaken of methods for the determination of the oil content of flaxseed and other oleaginous seeds.

Approved.

BAKING POWDERS AND BAKING CHEMICALS

It is recommended—

(1) That the qualitative test for aluminum, *Methods of Analysis,*

[1] *This Journal,* 14, 81 (1931).
[2] *Ibid.,* 15, 78 (1932).

A.O.A.C., 1930, p. 122, be dropped as official (final action). First action was taken in 1930.[1]

Approved.

(2) That the direct methods for the determination of available carbon dioxide be studied collaboratively with a view to limiting, insofar as practicable, the number of methods for this determination.

Approved.

(3) That further study be made of the tentative method for the determination of aluminum by its precipitation with phenylhydrazine.[2]

Approved.

(4) That the acetone method proposed by Cox or similar methods for the determination of free phosphoric acid in calcium acid phosphate be studied collaboratively.

Approved.

GUMS IN FOODS

It is recommended that the study of methods for gums be continued.

Approved.

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CHANGES IN THE OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE FORTY-EIGHTH ANNUAL CONVENTION, OCTOBER 31, NOVEMBER 1 AND 2, 1932[3]

I. SOILS

No additions, deletions, or other changes.

II. FERTILIZERS

(1) The first of the alternative methods for preparing ammonium citrate solution [p. 17, section 13 (1)] was deleted (first action). Accordingly the preceding sentence, "Prepare according, etc." was also deleted.

(2) The following method for the preparation of ammonium citrate solution was adopted as official (first action):

Ammonium citrate solution.—Should have a specific gravity of 1.09 at 20° and a pH of 7.0 as determined by the electrometric method with the hydrogen electrode or by the colorimetric method with phenol red. When using the colorimetric method proceed as follows:

Dissolve 370 g of crystallized citric acid in 1500 cc of H_2O and nearly neutralize by adding 345 cc of NH_4OH soln (28–29% NH_3). If the concentration of the NH_4OH is less than 28%, add a correspondingly larger volume and dissolve the citric acid in a correspondingly smaller volume of H_2O. Cool, and make exactly neutral as follows:

[1] *This Journal*, 14, 80 (1931).
[2] *J. Am. Chem. Soc.*, 47, 142 (1928).
[3] Compiled by Marian E. Lapp, *Associate Editor*. Unless otherwise stated, all references in this report are to *Methods of Analysis*, A.O.A.C., 1930, and the methods are edited to conform to the style used in that publication.

Transfer 10 cc of the citrate soln to one of the standard test tubes of a hydrogen-ion comparator set with color standards and add 0.5 cc of a 0.02% soln of phenol red or a sufficient volume to give the same concentration of indicator as used in the color standards. Add from a graduated pipet a few drops of NH₄OH (1+7), mix, compare the color by use of the comparator with that of the color standards of the same indicator, add more NH₄OH, if necessary, and repeat the test until the color matches that of the color standard corresponding to a pH of 7.0. If the NH₄OH added is in excess of that required to give a pH of 7.0, discard the soln and repeat the test, using a smaller quantity of NH₄OH. From the quantity of NH₄OH soln required to produce in the sample a color that exactly matches the standard, calculate the quantity of strong NH₄OH required to neutralize the rest of the solution. Add this calculated quantity of NH₄OH and check its reaction by repeating the test as before with the addition of a small quantity of NH₄OH or of a citric acid soln as may be required. When the color matches, dilute the soln, if necessary, to a density of 1.09 at 20° C. The volume will be about 2 liters. Keep in tightly stoppered bottles and check pH from time to time.

Phenol red is recommended in place of bromthymol blue as the salt effect due to the presence of the ammonium citrate solution gives a pH reading with the latter indicator that is approximately 0.20 unit too high. When bromthymol blue is used, a factor of 0.20 must be subtracted from the observed reading to give the true reading.

(3) The tentative method for the determination of chlorine (p. 30) was adopted as an official method (final action).

III. SEWAGE*

IV. AGRICULTURAL LIMING MATERIALS

No additions, deletions, or other changes.

V. AGRICULTURAL DUST*

VI. INSECTICIDES AND FUNGICIDES

(1) The following electrolytic method (Method II) for the determination of lead oxide and copper in Bordeaux-lead arsenate mixtures[1] was adopted as an official method (first action):

LEAD OXIDE AND COPPER IN BORDEAUX-LEAD ARSENATE MIXTURES

APPARATUS

Electrodes.—The cathode should be a cylindrical platinum electrode, either gauze or plate. The cylinder should be approximately 50 mm high and 25 mm in diameter. The anode may be made of gauze or plate. The cylinder should be approximately 50 mm high and 50 mm in diameter. This electrode should be sandblasted.

DETERMINATION

Weigh 1 g of the powdered sample and transfer to a 250 cc beaker. Add 15 cc of HCl and 5 cc of HBr (approximately 1.3 sp.gr.), and evaporate to dryness on a steam bath. Repeat the treatment, and finally, to remove the last traces of As, add 20 cc of the HCl and again evaporate to dryness.

To the residue add 25 cc of H₂O and 15 cc of HNO₃ and heat to boiling. Cau-

tiously boil until most of the bromides and some of the chlorides are expelled (characterized by changes in color, first from brown to green, and then to blue). Place the mixture on a steam bath and evaporate to dryness. Add 10 cc of H_2O and 15 cc of HNO_3, and again evaporate to dryness. Take up in 50 cc of H_2O and 12 cc of HNO_3, and heat until all salts are in solution. (It is not necessary to filter off any siliceous material that may be present.) Dilute to 200 cc and electrolyze overnight, employing a current of 0.15 ampere and a potential of 1.5 to 2 volts. Use electrodes of the type described under "Apparatus."

Add 15–20 cc of H_2O to the electrolyte and continue to use the current for a few minutes. If there is no further deposition on the newly exposed surfaces of the electrodes, wash them several times with H_2O without breaking the current. Finally break the current and wash once with methyl or ethyl alcohol. Dry the electrodes in an oven at 105–110° for 1 hour. The increase in weight of the cathode represents the Cu present in the sample, and the increase in weight of the anode represents the lead as PbO_2. From the increased weight of the cathode, calculate the percentage of Cu in the sample.

As the PbO_2 is not completely anhydrous, multiply the weight found by the factor 0.9267, and calculate the percentage of PbO in the sample.

VII. CAUSTIC POISONS

No additions, deletions, or other changes.

VIII. NAVAL STORES

No additions, deletions, or other changes.

IX. PAINTS

No additions, deletions, or other changes.

X. LEATHERS

No additions, deletions, or other changes.

XI. TANNING MATERIALS

No additions, deletions, or other changes.

XII. PLANTS

(1) In the official method for the determination of sand and silica (p. 102) a 10–50 g sample was adopted as official (final action).

(2) The heading "Ferric and aluminum oxides—Official" (p. 103) and the expression in parentheses, "Applicable to plant materials other than seeds," were deleted (final action).

(3) The heading "Manganese, calcium, and magnesium—Official" (p. 104) and the expression in parentheses "Applicable to plant materials other than seeds" were deleted (final action).

(4) The method for the determination of calcium (p. 104) was adopted as official (final action).

(5) The tentative micro method for the determination of calcium (p. 105) was adopted as official (first action).

(6) The method for the determination of magnesium (p. 106) was adopted as official (final action).

(7) The magnesium nitrate method for the determination of sulfur (p. 110) was adopted as official (final action).

(8) Method I for the determination of phosphorus (p. 110) was adopted as official (final action).

(9) Method II, the tentative micro method for the determination of phosphorus (p. 110) was adopted as official (first action).

(10) The method for preparation of plant materials for analysis (p. 102) was adopted as official (final action).

(11) The method submitted by the associate referee in 1930[1] for the determination of ammonia in tobacco was adopted as a tentative method.

(12) The method submitted by the associate referee in 1930[2] for the determination of free nicotine in tobacco was adopted as a tentative method.

XIII. FIBERS*

XIV. PAPER AND PAPER MATERIALS*

XV. BAKING POWDERS AND BAKING CHEMICALS

The official qualitative test for aluminum (p. 122) was deleted (final action). First action was taken in 1930.[3]

XVI. BEVERAGES (NON-ALCOHOLIC) AND CONCENTRATES

No additions, deletions, or other changes.

XVII. BEERS, WINES, AND DISTILLED LIQUORS

No additions, deletions, or other changes.

XVIII. COFFEE AND TEA

The directions in the method for the determination of caffeine in tea (p. 155) making the determination of nitrogen compulsory instead of optional (official, first action in 1930)[4] was made official (final action).

XIX. CACAO BEAN AND ITS PRODUCTS

(1) The method for the determination of crude fiber in milk chocolate adopted as official (first action) last year[5] was adopted as official (final action).

(2) The method for the determination of sucrose in chocolate published in the report of the associate referee last year[6] was adopted as a tentative method.

[1] *This Journal,* 14, 229 (1931).
[2] *Ibid.,* 231.
[3] *Ibid.,* 80.
[4] *Ibid.,* 81.
[5] *Ibid.,* 15, 72 (1932).
[6] *Ibid.,* 556.

(3) The method for the determination of lactose in milk chocolate published in the report of the associate referee last year[1] was adopted as a tentative method.

(4) The present tentative method for the determination of sucrose and lactose in cacao products (p. 158) was deleted.

XX. CEREAL PRODUCTS

(1) The following colorimetric method submitted by the associate referee for the determination of the hydrogen-ion concentration of flour was adopted as tentative.

Proceed as directed under 11, p. 167, to the last sentence. For the last sentence substitute the following: "Let stand quietly for 10 min, then decant the supernatant liquid into the colorimetric H-ion vessels, and immediately determine its hydrogen-ion concentration by comparison with suitable colorimetric standards."

(2) The vacuum method for the determination of moisture in air-dried bread (p. 177, par. 48) was made official (final action).

(3) The method for determining moisture in bread by heating at 130° for one hour (p. 177, 49 and p. 166, 5) was made official (final action).

(4) The vacuum method for determining moisture in air-dried bread and the method for determining moisture in bread by heating at 130° for one hour were made official, first action, for the determination of moisture in air-dried baked products other than bread.

(5) The method for the determination of crude fiber in bread and alimentary pastes (p. 178, 55 and 181, 66) was made official, final action.

(6) The official method for the determination of crude fiber in bread and alimentary pastes was made tentative for baked products other than bread.

XXI. COLORING MATTERS IN FOODS

No additions, deletions, or other changes.

XXII. DAIRY PRODUCTS

(1) The method submitted in 1929 by the associate referee for the determination of lactose and sucrose in cheese[2] was adopted as tentative after the following changes had been made: In line 8, "500 cc. volumetric flask" was substituted for "300 cc. volumetric flask"; line 10, "Transfer 100 cc. of the filtrate to each of two 200 cc. volumetric flasks" was changed to "Transfer 150 cc. of the filtrate to each of two 250 cc. volumetric flasks"; lines 14, 15 and 16 were changed to read as follows: "Determine the lactose in a 50 cc. aliquot as directed in 54, p. 383. Treat the contents of the other volumetric flask as directed in 23 (c), p. 373, using 10 cc. of hydrochloric acid, etc." Line 18, after the last sentence, the clause "Filter

[1] *This Journal*, 15, 558 (1932).
[2] *Ibid.*, 13, 243 (1930).

if necessary through a dry filter paper," was added. In line 7, p. 244, "factor 0.95" was changed to "factor 0.97."

(2) Method II, Albumin, section 11, p. 216, was deleted as an official method (final action).

XXIII. EGGS AND EGG PRODUCTS

(1) The tentative method for the determination of phosphoric pentoxide (p. 248) was adopted as official (first action) after the heading had been changed from "Phosphoric-pentoxide" to "Total Phosphoric Acid P_2O_5)", and reagent "(b) Olive oil" and the sentence "Add to the whites 0.5 cc of olive oil" (17, line 4), had been deleted.

(2) The method submitted by the associate referee for the determination of fat by acid hydrolysis[1] was adopted as tentative. The method for liquid eggs is as given in the preceding reference; for dried eggs proceed as follows:

Dried Eggs.—Transfer 1 g of the well-mixed sample into a Mojonnier fat extraction tube; add slowly, washing down any egg particles adhering to the sides of the tube, 10 cc of HCl (4+1); and proceed as directed under *Liquid Eggs.*

(3) The following extraction method for the determination of lipoids and lipoid phosphoric acid was adopted as tentative:

LIPOIDS AND LIPOID PHOSPHORIC ACID (P_2O_5)

REAGENTS

(a) *Mixed solvent.*—Equal volumes of $CHCl_3$ and absolute alcohol.

(b) *Alcoholic sodium hydroxide.*—Prepare a saturated soln free from carbonates by dissolving 100 g of NaOH in 100 cc of H_2O. Allow the mixture to stand until clear, or filter through a hard filter paper which has been soaked in alcohol. (5 cc of the NaOH soln contains approximately 4 g of NaOH.) Dissolve 50 cc of this soln in 900 cc of 95% alcohol and dilute with 95% alcohol to 1 liter.

PREPARATION OF SOLUTION

(a) *Liquid Eggs.*—Weigh accurately by difference approximately 4 g of the well-mixed sample into a 100 cc volumetric flask, add very slowly (drop by drop) from a pipet (shaking constantly until the proteins become coagulated and then thoroughly broken up) 25 cc of the mixed solvent, then 60–65 cc more of the solvent, and allow to stand 1 hour, shaking at 5 minute intervals. Fill to the mark with the solvent, shake, allow the mixture to stand until clear, and transfer a 50 cc aliquot to a 250 cc beaker.

(b) *Dried Eggs.*—Transfer 2 g of the well-mixed sample into a 100 cc volumetric flask, add 85–90 cc of the mixed solvent, and allow to stand 1 hour, mixing at 5 minute intervals. Proceed as directed under *Liquid Eggs.*

DETERMINATION

Lipoids.—Transer 50 cc of the filtrate prepared as in (a) to a 150 cc beaker, and evaporate the extract to dryness on a steam bath. (An electric fan or a gentle blast of dry air may be used to hasten evaporation.) Place the beaker into an oven at 100° for 5–10 min to remove any remaining moisture. Dissolve the dry extract in 5–10 cc of $CHCl_3$, and filter the solution into a weighed 100 cc Pyrex beaker through

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[1] *This Journal,* 15, 313 (1932).

a pledget of cotton packed into the stem of a 'funnel, transferring all soluble extract from the bottom and sides of the beaker by means of $CHCl_3$ from a wash bottle. Finally wash the funnel and stem tip. (The filtrate should be clear.) Evaporate the $CHCl_3$ on a steam bath, and dry the beaker and contents in an oven at 100° to minimum weight (approximately 90 min). Allow the beaker to stand in the air until no further change in weight takes place (approximately 30 min), weigh, and report as percentage of lipoids.

Lipoid Phosphoric Acid (P_2O_5).—Dissolve the dried lipoids in 2–3 cc of $CHCl_3$, add 10–20 cc of the alcoholic NaOH soln, evaporate to dryness on a steam bath, using care to avoid spattering, and place the beaker into an oven at 100° for 30 min to remove any remaining moisture. Transfer the beaker while hot to an electric muffle heated to 500° (faint redness), and allow it to remain at that temp. for 1 hour. Cool, add a few drops of H_2O, break up the charge with a glass rod (flattened end), cover the beaker with a watch-glass, add slowly 5 cc of HNO_3 (1+3), mix, remove the watch-glass, and filter, collecting the filtrate in a 300 or 500 cc Erlenmeyer flask. Wash thoroughly the charred material and filter paper with H_2O from a wash bottle.

In the prepared filtrate determine phosphoric acid (P_2O_5) as directed under II, 10 (b), (p. 16), using 20–50 cc of the molybdate soln. Report as percentage.

(4) The method submitted in 1930[1] for the determination of reducing sugars and sucrose was adopted as official (first action).

(5) The part of the tentative method for the determination of water-soluble nitrogen and water-soluble nitrogen precipitable by 40 per cent alcohol (crude albumin nitrogen) applicable to liquid eggs[2] was adopted as official (first action) and the title of the method was changed to "Crude Albumin Nitrogen."

(6) The tentative method for the determination of chlorine (p. 249) was adopted as official (first action) after the reagent "(b) Olive oil" had been deleted.

(7) The quality test for glycerol, published in the 1931 report of the associate referee,[3] was adopted as tentative after the last sentence had been changed to the following: "In the presence of glycerol (due to acrolein) a strong pink color develops within 1 min and becomes a deep violet-red within 5 min."

(8) The method published in the report of the associate referee in 1931 for the quantitative determination of glycerol,[4] with the heading "Glycerol (not applicable in the presence of added sugars)," was adopted as tentative.

(9) Owing to the recent compilation of extensive data resulting from work done on eggs, sections 23 and 24 (p. 249) were deleted.

(10) Sections 16, 17, 18, 19, 20, 21, and 22, pp. 248–50, were changed to sections 14, 15, 16, 17, 18, 19, and 20, respectively, and the sections at present numbered 14 and 15, were changed to sections 21 and 22, respectively.

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[1] *This Journal*, 14, 397 (1931).
[2] *Ibid.*, 15, 75 (1932).
[3] *Ibid*. 381.
[4] *Ibid.*, 334.

XXIV. FISH AND OTHER MARINE PRODUCTS*
XXV. FLAVORING EXTRACTS

(1) Methods I and II, previously published,[1] for the determination of essential oil in extracts and toilet preparations were adopted as tentative methods after the following corrections had been made: The general heading was changed to read: Essential Oil in Extracts and Toilet Preparations; that of the first part to read: *Method I*—(Applicable to extracts of anise, lemon, nutmeg, orange, rosemary, thyme, wintergreen, and methyl salicylate); and that of the second part to read: *Method II*—(Applicable to extracts of cinnamon and cloves.) For both methods standardized Babcock bottles and 10 cc burets to measure the solvent were specified.

XXVI. FRUITS AND FRUIT PRODUCTS

The word "official" was deleted from section **49**, p. 276.

XXVII. GRAIN AND STOCK FEEDS

No additions, deletions, or other changes.

XXVIII. MEAT AND MEAT PRODUCTS

The following method for the determination of salt was adopted as a tentative method:

SALT

Moisten 2-1/2–3 g of the finely comminuted and thoroughly mixed sample in a platinum dish with 20 cc of 5% sodium carbonate soln, evaporate to dryness, and ignite at a temp not exceeding dull redness. Extract with hot H_2O, filter, and wash. Return the residue to the platinum dish and ignite to an ash. Dissolve the ash in HNO_3 (1+4), filter to free from any insoluble residue, wash thoroughly, and add the wash soln to the H_2O extract. Determine Cl in the combined filtrate and washings as directed on p. 111, **34** and **35**.

XXIX. METALS IN FOODS

The following bromate method for the determination of arsenic on fresh fruits was adopted as tentative:

Bromate Method

(Applicable to the determination of arsenic in plants and food products where a sample of convenient size for digestion will yield at least
0.005 grain (0.324 mg) of As_2O_3.)

REAGENTS

(a) *Nitric acid.*—As-free and containing not less than 67% HNO_3.

(b) *Sulfuric acid.*—As-free and containing not less than 93% H_2SO_4.

(c) *Hydrochloric acid.*—As-free and containing not less than 35% HCl.

(d) *Ammonium oxalate-urea soln.*—To a saturated H_2O soln of ammonium oxalate add 50 g of urea per liter.

(e) *Sodium or potassium bromide.*—C. P.

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[1] *This Journal*, 15, 539 (1932).

(f) *Ferrous sulfate.*—C. P.

(g) *Sodium chloride.*—Commercial salt, uniodized.

(h) *Methyl orange indicator.*—Dissolve 0.5 g in H_2O and dilute to 1 liter.

(i) *Standard potassium bromate soln.*—Dissolve 0.1823 g in H_2O and dilute to 1 liter. One ml is equivalent to 0.005 grain of As_2O_3. Check by titration against standard arsenious oxide soln, making the titration at about 90° and in the presence of about 100 ml of water and 25 ml of HCl in order to simulate the conditions under which the samples will be titrated. One ml of the bromate soln should be equivalent to 1 ml of the As_2O_3 soln.

(j) *Standard arsenious oxide soln.*—Dissolve 0.3241 g of As_2O_3 in 25 ml of 10% NaOH, make slightly acid with H_2SO_4 (1+6), and dilute with H_2O to 1 liter.
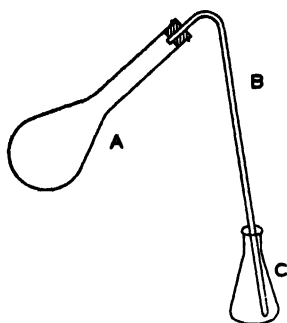
DISTILLING APPARATUS

The distilling apparatus consists of a Kjeldahl, flask, 800 ml (A), distilling tube (B), and Erlenmeyer flask, 300 ml (C).

To prepare the distilling tube, bend a 10–15 mm glass tube to an acute angle of about 70°. Draw the long arm, which is about 15–20 inches long, down to an orifice of about 3 mm. Fit the short arm (about 4 inches long) with a No. 7 rubber stopper which has previously been boiled in 10% NaOH for 15 min, and then in HCl for 15 min, in order to remove most of the sulfur compounds which might be distilled and react with the bromate soln.

DISTILLING APPARATUS

PROCEDURE

Acid Digestion.—Introduce a suitable sample containing 0.005 grain or more of As_2O_3 into an 800 ml Kjeldahl flask. (In case of fresh fruits such as apples and pears, use the peel from 1 pound, in which case the number of ml of bromate soln used, divided by 200, equals the grains of As_2O_3 per pound.) Proceed with the acid digestion as directed in *Methods of Analysis, A.O.A.C.*, 1930, 307, **3**, with the following exceptions: Commence with only 35–50 ml of HNO_3 to minimize bumping; use exactly 20 ml of H_2SO_4, or, if the material is difficult to digest, exactly 25 ml. Heat over an asbestos mat with a 3 inch hole.

Removal of Oxides of Nitrogen.—After digestion is complete, add either 50 ml or 75 ml of reagent (d) according to whether 20 ml or 25 ml of H_2SO_4 has been employed during digestion. Boil until the white SO_3 fumes extend well up in the neck of the flask.

Distillation of $AsCl_3$.—Add 25 ml of H_2O to the digested soln in the Kjeldahl flask and cool to room temp. Put 100 ml of H_2O into the Erlenmeyer flask. Have ready the following mixture: 0.5 g of KBr (or NaBr), 2 g of $FeSO_4 \cdot 7H_2O$, and 30 g of NaCl, add to the digested soln in the Kjeldahl flask, also 25 ml of HCl, and connect the distilling tube. Heat the Kjeldahl flask over a small, well-protected flame, and distil into the H_2O in the Erlenmeyer flask. (The heating is not intended to boil the soln but to bring about the evolution of the HCl gas, which carries over the $AsCl_3$ with it. The absorption of the evolved HCl gas by the water causes a rise in temperature which furnishes an indication of the progress of the distillation.) Adjust the flame so that the temp. of the distillate soln will rise to 90° in 9–11 min.

Titration of the $AsCl_3$.—Titrate the distillate at once with the bromate soln, using 3 drops of methyl orange indicator. Single drops of indicator, but not ex-

ceeding 3, may be added during titration as the red color fades. Towards the end of the titration add the bromate soln very slowly and with constant agitation to prevent local excess. The end point is reached when a single drop of the bromate just destroys the final tinge of red color. To determine when this point has been reached, use an Erlenmeyer flask of clear water for comparison. The end point must not be exceeded as the action of the indicator is not reversible and back titrations are not reliable. At the proper end point, the red color produced by 2 additional drops of methyl orange indicator should persist for at least 1 min. Correct for the volume of bromate used in a blank run with the same reagents and the regular distillation procedure. The blank titration should not exceed 0.7 ml of bromate soln. The method is accurate down to the variations in the blank which should not exceed 0.1 ml when chemicals from the same lot are used. Should the blank titration be high or variable, test the individual reagents for purity by titration. The H_2SO_4 should furnish most of the blank. To test this reagent, bring 20 ml to a boil, cool, dilute with H_2O to 100 ml, add a little HCl, and titrate while hot.

XXX. NUTS AND NUT PRODUCTS*

XXXI. OILS, FATS, AND WAXES

(1) The vacuum oven method for the determination of moisture and volatile matter[1] was made official (final action).

XXXII. PRESERVATIVES AND ARTIFICIAL SWEETENERS

(1) The following clause was inserted in the first line, p. 345, after the word "hour": (1–1/2 hours in case of dried fruits).

(2) The following parenthetical expression was inserted under the heading "Monier-Williams Method—Tentative": (Applicable in the presence of other volatile sulfur compounds), and the position of this method, **34**, was changed in order to have it follow section **31**, p. 344, and be included under the heading "Total Sulfurous Acid."

(3) The directions of the method for the quantitative determination of boric acid, **16**, p. 340, were changed to provide for the use of 1–2 grams of mannitol instead of 10 grams for the titration (official, first action).

(4) The change from 10 per cent to 1 per cent solution of $CuSO_4$, line 4, section **3**, p. 335 was made official (final action).

XXXIII. SPICES AND OTHER CONDIMENTS

(1) The following tentative methods: Determination of total solids (p. 355, **36**); of total acid (**40**); of oil (p. 356, **41**); of lecithin P_2O_5 (**43**); and of egg solids (**44**) were deleted.

(2) The following method for the determination of total solids was adopted as official (first action):

TOTAL SOLIDS

Use a 2 g sample and proceed as directed in **2**, p. 244.

(3) The following method for the determination of total acidity was adopted as an official method (first action).

[1] *This Journal*, 15, 78 (1932).

TOTAL ACIDITY

Weigh about 15 g into a 500 cc Erlenmeyer flask, dilute to about 200 cc, and shake until all lumps of dressing are thoroughly broken up. Titrate with 0.10 *N* NaOH, using neutral phenolphthalein, and calculate as acetic acid. In order to recognize the end point, have a duplicate sample at hand so that, by comparison, the first change of color may be noted.

(4) The following method for the determination of total nitrogen was adopted as official (first action):

TOTAL NITROGEN

Weigh about 15 g into a 500 cc Kjeldahl flask and place on the steam bath until the egg is thoroughly cooked and the oil separates readily. Cool and add about 50 cc of petroleum ether, mix, and pour off the ether solution through a small filter. Repeat the ether treatment twice, rinsing out as much oil as possible. Wash the filter with petroleum ether and add the filter paper to the sample in the flask. Determine nitrogen, using 35 cc of sulfuric acid for digestion, as directed in 5, p. 245. (The contants given in these formulas are the average fat of yolk, average solids of yolk, and average solids of white given by Mitchell for commercially fresh shell eggs.)

(5) The following method for the determination of total phosphoric acid (P_2O_5) was adopted as official (first action):

TOTAL PHOSPHORIC ACID (P_2O_5)

Use a 10 g sample and proceed as directed in 16, p. 248, except to use a platinum dish in place of the beaker and to burn off the oil before ashing in the muffle.

(6) The following method for the determination of total fat was adopted as tentative:

TOTAL FAT

Use a 2 g sample and proceed as directed under "Fat (by hydrolysis)."[1]

(7) The following procedure for calculating composition was adopted as tentative:

CALCULATION OF COMPOSITION

When $P = \%$ total P_2O_5 and $N = \%$ total nitrogen, then
% yolk $= 75.69\ P - 1.802\ N$;
% white $= 60.80\ N - 114.59\ P$;
% total egg $=$ yolk $+ \%$ white;

$$\% \text{ white in egg component} = \frac{\% \text{ white}}{\% \text{ total egg}} \times 100;$$

Vegetable oil $=$ total fat $-$ (yolk $\times 0.3188$);
Vinegar (4% acid strength) $=$ total acidity as acetic $\times 25$;
Minor constituents (sugar, salt, spices, stabilizers) $=$ total solids $-$ (yolk $\times 0.5047$) $-$ (white $\times 0.1221$) $-$ vegetable oil; and
Added water $= 100\% -$ total egg $-$ vegetable oil $-$ vinegar $-$ minor constituents.

XXXIV. SUGARS AND SUGAR PRODUCTS

(1) The following sentence was added to Section 18 (a), p. 368: "This reagent is used primarily for clarifying dark colored cane, sorghum, and

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[1] *This Journal*, 15, 313 (1932).

beet products when sucrose is determined by polariscopic methods" (official, first action).

(2) The following sentence was added to section **18 (b)**, p. 368: "Alumina cream is suitable for clarifying light-colored sugar products or as an adjunct to other agents when sugars are determined by polariscopic or reducing sugar methods" (official, first action).

(3) The last sentence in section **18 (d)**, p. 369, was changed to read as follows: "This reagent may be used for clarifying light-colored sugar products when sugars are determined by polariscopic methods, and its use is imperative when sugars are determined in the solution used for polarization" (official, first action).

(4) Under section **18 (e)**, p. 369, the following sentence was inserted before the last sentence: "This reagent is used for the same purposes as the one described under **18 (a)**," (official, first action).

(5) The first part of the text of section **22**, p. 371 was changed to read as follows:

(a) *Direct Reading;* Dissolve the double normal weight (52 g) or a fraction thereof, of the substance in H_2O in a 200 cc volumetric flask; add the necessary clarifying agent [**18 (a), (b), (d),** or **(e)**], avoiding any excess; shake; dilute to the mark with H_2O; mix well and filter, keeping the funnel covered with a watch-glass. Reject the first 25 cc of the filtrate. If a lead clarifying agent was used, remove the excess lead from the solution when sufficient filtrate has collected by adding anhydrous Na_2CO_3, a little at a time, avoiding any excess; mix well and filter again, rejecting the first 25 cc of the filtrate.—"

(6) The sentence beginning "Repeat this procedure" section **103, 2,** p. 391, was changed to read as follows: "Repeat this procedure from time to time until a reading is obtained corresponding to 64.5% solids (N_{20} = 1.4521) or to such other value as in the experience of the analyst will give a filtered sirup of 65.0% solids."

(7) In the directions for the preparation of sample for maple sugar and other solid or semi-solid products, section **103 (b), 2,** the first sentence was amended to read as follows: "Prepare a sirup by dissolving approximately 100 g of the sample in 150 cc of hot H_2O, boil until the temp. approaches 104°, and complete the preparation of the resulting sirup as directed in **(a)** 2, commencing at "draw a small quantity into a thin-walled pipet.""

(8) The following sentence was added to the official method for the determination of moisture in maple sirup (p. 391, **104**): "In the refractometric measurement guard against deposition of dew on the prisms by circulating water through the prism jackets and correcting the observations to 20° by use of Table XLII, 7" (official, first action).

(9) The heading "Sucrose in the absence of raffinose" (p. 392, **107**) was deleted, and the directions were changed to read as follows: "Calculate from the results of **105**, using the appropriate formula from **22** or **23**" (official, first action).

(10) The directions for preparing the reagent used in the determination of the Winton lead number were changed to read as follows:

Standard basic lead acetate soln.—To a measured volume of the reagent prepared for determination of the Canadian lead value (118), add 4 volumes of H_2O, and filter. A blank (116) should be run with each set of determinations" (official, first action).

(11) The directions for preparation of the reagent for the determination of the Canadian lead number (Fowler modification, tentative), p. 393, 118, were changed to the following:

Standard basic lead acetate soln.—Activate litharge by heating it to 650–670° for 2.5–3 hours in a muffle. (The cooled product should be lemon color.) In a 500 cc Erlenmeyer flask provided with a return condenser boil 80 g of normal lead acetate crystals and 40 g of the freshly activated litharge with 250 g of H_2O for 45 min. Cool, filter off any residue, and dilute with recently boiled H_2O to a density of 1.25 at 20° (official, first action).

(12) The directions for determining conductivity value (official, first action), p. 393, 120 and 121, were revised to read as follows:

<div align="center">APPARATUS</div>

120 (a)

(1) *Conductivity cell.*—Should be made of resistance glass with platinized platinum electrodes firmly fixed and adequately protected from displacement. These electrodes may be sealed into a vessel into which the solution under examination may be run and subsequently drawn off (Zerban type), or attached to a support so that they can be lowered into a cylinder (or a 100 cc beaker) containing the solution (dipping type). The cell must be provided with a thermometer graduated in tenth of degrees and covering the range of 20–30°, and the bulb must be placed in the immediate vicinity of the electrodes. The cell constant should be approximately 0.15.

(2) *Galvanometer.*—Or a microphone hummer (or an induction coil) and a sensitive telephone receiver.

(3) *Suitable source of current.*—Dry or storage cells if a hummer or induction coil is used; 110 volt alternating current if a galvanometer is used.

(4) *Resistances of 10 and 100 ohms.*—Should be fixed and accurate.

(5) *Slide wire or Wheatstone bridge.*

(6) *Device for control of the temperature of the cell to within ±0.1°.*—This may consist of a thermostat or of a vessel into which H_2O of suitable temp. may be run so as to adjust the cell contents to 25°.

<div align="center">DETERMINATION OF THE CELL CONSTANT</div>

120 (b)

Prepare solns of 0.3728 and 0.7456 g of dry C. P. KCl in H_2O, which offers a resistance of at least 25000 ohms in the cell, and make them to the mark at 20–25° in 500 cc volumetric flasks. Fill the cell with the more dilute (0.01 M) soln, adjust to 25°±0.1°, measure the electrical resistance, and multiply the number of ohms by 141.2. Rinse with the stronger (0.02 M) soln, fill the cell with this soln, measure its resistance at 25°, and multiply by 276.1. Average the two results.

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Weigh out a quantity of sirup that contains 25 g of dry matter, transfer to a 100 cc volumetric flask with warm H_2O of the same quality as that used in the determination of the cell constant, cool to 25°, make to mark, and measure the resistance in the cell at 25° ±0.1°. Divide the cell constant by the number of ohms found.

(13) The official refractometric method, **7**, p. 365, was amended by the insertion of the following sentence in the sixth line after the word "soln" (official, first action).

(If the determination is made at temps appreciably above 20°, or if the humidity causes condensation of moisture on the exposed faces of the prisms, make the measurements at room temp and correct the readings to the standard temp of 20°.)

XXXV. VEGETABLES AND VEGETABLE PRODUCTS

No additions, deletions, or other changes.

XXXVI. VITAMINS*

XXXVII. WATERS, BRINE, AND SALT

No additions, deletions, or other changes.

XXXVIII. RADIOACTIVITY OF FOODS AND DRUGS

No additions, deletions, or other changes.

XXXIX. DRUGS

(1) The following method for the determination of cascara sagrada was adopted as tentative:

CASCARA SAGRADA

APPARATUS

Type C Continuous Extractor.[1]

REAGENTS

(a) *Acetic acid soln.*—(1+100).

(b) *Sulfuric acid soln.*—(1+1).

(c) *Sodium bicarbonate soln.*—(5+100). Make up in cold H_2O as needed; add 1 cc of 0.1 N HCl to insure freedom from carbonates.

(d) *Sodium carbonate soln.*—Saturated.

(e) *Hydrochloric acid.*—(1+1).

Introduce $CHCl_3$ into the continuous apparatus to within 5 cm of the overflow. Adjust a 200 cc Erlenmeyer flask carrying 125 cc of $CHCl_3$ to the apparatus with a well-fitted, tin-foiled cork. Into the inner tube of the apparatus introduce a measured or weighed portion of the sample representing approximately 2 g of cascara sagrada. Add 20 cc of H_2O and 1 cc of reagent (a) to the cascara layer. Connect the apparatus to the condenser. (The outlet of the condenser should not be constricted. If it is, place a hole in the side near its tip to insure free return of $CHCl_3$.)

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[1] *Ind. Eng. Chem.*, **17**, 612 (1925).

Adjust the burner (use an asbestos ring to prevent overheating) and reflux rapidly for 2 hours. (At this time the CHCl₃ in the tube will be colorless.) Disconnect the flask and discard its contents.

Recharge the Erlenmeyer flask with 125 cc of CHCl₃ and connect to the apparatus, which still carries the CHCl₃-exhausted acetic acid soln of the original sample and the clear exhausted CHCl₃. Add 10 cc of reagent (b) to the cascara layer by means of a pipet.

Connect the apparatus to the condenser, adjust the burner, and reflux rapidly. At the end of the 3 hours, the CHCl₃ in the apparatus should be practically colorless (it may contain a small amount of color, a non-emodin material).

Remove the flame and disconnect the flask. Transfer the CHCl₃ in the flask to a separator, wash the flask with 10 cc of H₂O, and transfer the H₂O to a separator carrying CHCl₃. Shake, withdraw the CHCl₃, and again wash the H₂O with 10 cc of CHCl₃, adding the washings to the main CHCl₃ soln. Wash the CHCl₃ with three 10 cc portions of reagent (c), then wash the combined reagent (c) with CHCl₃ two or three times. Discard the aqueous soln.

Shake out to exhaustion the combined CHCl₃ with reagent (d) in a train of separators. (Four 10 cc portions should suffice.) Wash the combined reagent (d) with CHCl₃ several times. Discard all the CHCl₃.

Add sufficient reagent (e) to the aqueous soln (cautiously, a few cc's at a time) to insure an acid reaction. Extract with CHCl₃ to completion. (Three 20 cc portions should be sufficient.) Combine the CHCl₃ and wash with 5 cc of H₂O. Filter the CHCl₃ through a filter wetted with CHCl₃. Evaporate to 20 cc. Transfer the residue to a small glass or platinum dish, evaporate to dryness, and dry at 100° for 2 hours. Cool, and weigh.

(2) The method submitted by the associate referee in 1931[1] for the determination of aloin was adopted as tentative.

(3) The following tests and descriptions for nicotine and sparteine were adopted as tentative:

NICOTINE AND SPARTEINE
REAGENTS

(a) *Mercuric chloride soln.*—5%.

(b) *Mercuric chloride—sodium chloride soln.*—(1) Dissolve 5 g of HgCl₂ and 0.75 g of NaCl in 100 cc of H₂O.

(c) *Gold chloride soln.*—5%.

PREPARATION OF SAMPLES

Controls.—Dissolve 1 mg of the pure alkaloidal salt in two drops of H₂O to make approximately a 1:100 soln.

IDENTIFICATION

Place a drop of the alkaloidal soln on a clean glass slide; add a drop of reagent by means of a clean glass rod; and, without stirring or covering, examine under the microscope, using low power. (A magnification of 100 to 180 is suitable.) Note the kind of crystals formed and compare their characteristics with the description given and then with a control.

[1] *This Journal,* 15, 407 (1932).

Characteristics of Microchemical Tests for Alkaloids

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Nicotine	Mercuric chloride	Radiating transparent blades form in presence of slight excess H_2SO_4: feather blades form in the presence of HCl.
	Mercuric chloride-sodium chloride	Radiating transparent blades
Sparteine	Gold chloride	Large numbers of blade-like crystals varying in size according to concentration

(4) The following method for the determination of phenolsulfonates was adopted as tentative:

PHENOLSULFONATES

Dissolve the sample (equivalent to about 0.8 g of phenolsulfonate) in about 30 cc of H_2O, add 5 cc of HCl, and assay 10 cc portions with 0.1 N $Na_2S_2O_3$). (The bromine will be absorbed very rapidly at first, but as the titration proceeds the absorption becomes slower and slower.) Titrate as far as possible with no other indicator than the fading of the bromine yellow. (Usually this will be within about 1–4 cc of the end point.) Then use methyl orange (0.1%), a drop at a time, adding no new indicator until the previous drop has practically faded. After adding bromine soln, always wait a sufficient time for the absorption of the bromine before adding more methyl orange (10 seconds at first, 15 seconds at end of titration). In the presence of dibromphenolsulfonic acid, the action of bromine on methyl orange is much slower than normally, so it is necessary to wait a few seconds after each addition of bromine to see whether it is acting on the indicator or not. The end point is reached when, after waiting 15 seconds for the absorption of the last drop of bromine and adding a drop of methyl orange, the latter fades very appreciably in 10 seconds. It is always best, after the methyl orange has faded, to add another drop of the same to be sure that the first drop was not added too soon.

In using 0.1 N bromine, draw the indicator by dropping the tube from a 10 cc cylinder. If less than 1 cc of indicator is used, make no correction; if more, subtract 0.5 cc of 0.1 N Br for each cc of indicator.

1 cc of 0.4 N bromine = 0.02322 g of sodium phenolsulfonate;
1 cc of 0.1 N bromine = 0.0058035 g of sodium phenolsulfonate.

(5) The following method for the determination of sulfonal and trional was adopted as tentative:

SULFONAL AND TRIONAL

Mix about 0.5 g of the sample, accurately weighed, with pure clean sea sand and place the mixture in a Knorr tube containing a half-inch layer of asbestos. Using a bell jar and vacuum, extract the mixture with 10 portions of 10 cc each of ethyl ether, mixing the sample with the sand by means of a glass rod before each addition of ether. Collect the ether extractions in a tared flask, distil off the bulk of the ether, and allow the remaining solvent to evaporate spontaneously, rotating the flask to aid the evaporation. Dry the residue in a desiccator over H_2SO_4 acid for 18 hours and weigh. Identify the residue by means of its melting point.

If desired, the extraction may be carried on in a suitable automatic extraction apparatus.

(6) The following method for the determination of ipomea and jalap was adopted as tentative:

IPOMEA AND JALAP

Place 10 g of the drug in a No. 60 powder in an Erlenmeyer flask of about 250 cc capacity and add 50 cc of alcohol. Fit the flask with a stopper through which is inserted a glass tube about 1 m long to act as a reflux condenser, and heat the mixture on a gently simmering steam bath for 30 minutes, shaking occasionally. Transfer the contents of the flask to a small percolator and percolate slowly with warm alcohol until about 95 cc of tincture has been obtained. (To ascertain whether extraction is complete, collect a further 10 cc of percolate and pour a few drops into cold H_2O; if more than a faint cloudiness appears, continue the percolation with warm alcohol until the test for resin fails. Concentrate the additional percolate by evaporation and add the residue to the flask before making up to volume.) Cool the percolate to room temp and make up the soln to 100 cc with alcohol. Mix well.

Evaporate 25 cc of the tincture prepared as described (representing 2.5 g of drug) to dryness on the H_2O bath in a beaker or flask of suitable size and dry the residue until it is free from alcohol. Add 15 cc of H_2O, bring the mixture to boiling, allow to cool about 3 min, and stir well with a flat-headed glass rod for 2 min to insure thorough washing of the resin. Cool the mixture by placing the container in a jar of ice-cold water and decant the wash H_2O into a 9 cm filter paper. Repeat the washing of the resin with another 15 cc portion of H_2O, boiling and cooling the mixture, kneading the resin as before, and decanting the washings onto the filter, as described previously. Repeat the washing and kneading process with hot H_2O a third time. Dissolve the residue in the container in 10 cc of warm alcohol and pour the soln onto the filter, collecting the filtrate in a weighed beaker or flask. Use sufficient hot alcohol in small portions to completely transfer the soln of the resin to the filter and insure thorough washing of the filter. Evaporate the combined filtrate and washings to apparent dryness, add 1 cc of absolute alcohol, and evaporate the solvent, taking care to rotate the container in an inclined position as the last portions of the solvent are dissipated. Dry the residue at 80° to constant weight.

(7) The following microchemical methods for the determination of chinosol, benzocaine, pyridium, and cinchophen were adopted as tentative methods:

CHINOSOL, BENZOCAINE, PYRIDIUM, AND CINCHOPHEN

REAGENTS

(a) *Gold chloride.*—Dissolve 1 g of reagent gold chloride in 20 cc of H_2O.

(b) *Potassium thiocyanate.*—Dissolve 5 g of KSCN in 100 cc of H_2O.

(c) *Potassium ferrocyanide.*—Dissolve 5 g of $K_4Fe(CN)_6 \cdot 3H_2O$ in 100 cc of H_2O.

(d) *Magnesia mixture.*—Dissolve 55 g of $MgCl_2 \cdot 6H_2O$ and 140 g of NH_4Cl in H_2O, add 130.5 cc of NH_4OH and H_2O to make 1 liter.

PREPARATION OF SAMPLE

Separate the compound for microchemical testing in pure form by the use of suitable solvents.

Prepare a soln of 1–100 to 1–1000 concentration with the aid of acid, alkali, or H_2O as specified for individual synthetics.

PREPARATION OF CONTROL

Prepare a soln of the pure synthetic in the same concentration as the sample to be tested.

To a drop of a soln of the compound on a clean glass slide, add a drop of reagent, and without stirring or covering examine under the microscope. (A magnification of 100 to 150 is suitable.) Note the characteristics of the crystals formed and compare with control and description.

SYNTHETIC	METHOD OF SOLUTION	CONCENTRATION	REAGENT	DESCRIPTION OF CRYSTALS
Benzocaine	Dissolve in HCl. Avoid excess acid	1:100	Potassium ferrocyanide	Colorless irregular plates and rods
Chinosol	Dissolve the salt in H_2O. If free base, dissolve in HCl. Avoid excess acid	1:500	Magnesia mixture	Small elliptical grains. Few burr-shaped crystals on standing
Cinchophen	Dissolve in 0.1 N NaOH, add H_2O, and make slightly acid with HCl	1:1000	Gold chloride	Dark clusters of needles. Few short rhombic crystals
Pyridium	Dissolve the salt in H_2O. If free base, dissolve in HCl. Avoid excess acid	1:1000	Potassium thiocyanate	Small red-brown dense sheaves

XL. BACTERIOLOGICAL METHODS*

XLI. MICROCHEMICAL METHODS*

XLII. TABLES

No additions, deletions, or other changes.

REPORT OF THE COMMITTEE ON STANDARD SCALE FOR IMMERSION REFRACTOMETERS

In last year's report C. F. Snyder, Associate Referee on Drying, Densimetric and Refractometric Methods, pointed out that there is considerable difference in refractive index values corresponding to the same scale division on the immersion refractometers manufactured by two different companies. Both companies fit their instruments with arbitrary scales graduated in 110 divisions, numbered from −5 to +105. The refractive indices corresponding to the scale readings differ in the two instruments because the optical properties of the prisms vary. Thus the tables furnished to correlate refractive index with the arbitrary scale

units cannot be used interchangeably with the two instruments. In view of the confusion which has arisen from the introduction of these two scales, it is desirable that these instruments be standardized. Therefore, as recommended by Associate Referee Snyder, a committee was appointed by the Association to study this question.

It was suggested by the manufacturers that within certain limits it is possible to subject the optical glass to a preliminary heat treatment in such manner as to impart permanent changes in its optical properties, including the refractive index. Thus prisms could be made to conform to an arbitrary scale. The committee was informed that for instruments having a single prism there is no particular difficulty involved. The same conclusion is obviously true for the first prism on instruments designed for a series of interchangeable prisms. With the succeeding prisms of the series the problem becomes more difficult.

The committee was also informed that although the instruments theoretically could be graduated directly in refractive index units, which would entirely solve the problem, such a scale would greatly increase the labor of working with the instrument, particularly as regards reading and recording the scale values, in view of the fact that values to the fourth decimal place would be indicated. Such a procedure would present added difficulties for instruments with interchangeable prisms.

Because it is apparently possible to standardize an arbitrary scale suitable for immersion refractometers and because such an arbitrary scale is desirable from the standpoint of the user and the manufacturer, a decision must be made regarding the scale to be adopted. The question which naturally arises is whether the old or the new, or an entirely different scale, should be adopted. It is the opinion of the committee that the original arbitrary scale adopted by the Zeiss Optical Co. should be selected as the standard scale of this Association for the first prism because the Zeiss instrument was the only one of its type on the market for many years and therefore was naturally adopted by chemists and analysts as standard, and as a result considerable literature has accumulated expressing data only in terms of the Zeiss arbitrary scale units. Practically all this work is confined to the single prism covering the range of indices of 1.32 to 1.36. The committee therefore recommends that the so-called Zeiss scale be adopted as tentative for the prism having the range 1.32 to 1.36. This attitude on the part of the committee may seem to place an imposition upon our domestic manufacturer of immersion refractometers, but it is believed that the benefits to be derived from the adoption of a standard scale, by laying a more secure foundation for refractometers in analytical and industrial work, are sufficiently great to warrant this stand.

It may be mentioned that some of the older instruments of domestic manufacture are graduated according to the Zeiss scale and that the

more recent ones differ from the Zeiss scale. The committee is informed by the Bausch & Lomb Optical Company that immersion refractometers of their manufacture bearing serial numbers under 4000 are identical with the original Zeiss instrument with respect to calibration. Instruments whose serial numbers lie between 4000 and 10,000 have the modified scale. The same firm states further that instruments with serial numbers above 10,000 will conform to the original scale within the experimental accuracy of the instrument. To permit the use of those instruments graduated on the basis of the modified scale (serial numbers 4,000 to 10,000), in conjunction with tables given in units on the Zeiss scale, the following table is presented:

SCALE READING B & L MODIFIED SERIAL NOS. 4000 TO 10,000	REFRACTIVE INDEX	SCALE READING ZEISS
−5	1.32539	−5.0
0	1.32737	0.0
+5	1.32934	5.1
10	1.33131	10.1
15	1.33326	15.2
20	1.33521	20.2
25	1.33714	25.2
30	1.33907	30.3
35	1.34098	35.3
40	1.34289	40.4
45	1.34478	45.4
50	1.34667	50.5
55	1.34855	55.5
60	1.35041	60.5
65	1.35227	65.6
70	1.35411	70.6
75	1.35595	75.7
80	1.35778	80.8
85	1.35959	85.8
90	1.36139	90.8
95	1.36319	95.9
100	1.36497	100.9
105	1.36674	106.0

It can be assumed that many present-day users of refractometers are unaware of the difference which exists between the two immersion refractometers now on the market, and that this condition might result in considerable error or confusion if the data of the former investigators were interpreted by the use of the wrong conversion tables.

In regard to the question of a standard scale for the remaining prisms in the series, for indices above 1.36, the committee feels that further study should be made. It should be stated that the Bausch and Lomb system of

six prisms permits readings up to 1.54581, while the highest index readable on the Zeiss six-prism system is 1.49183.

The committee wishes to state that it has received close cooperation from the manufacturers of immersion refractometers.

Much of the existing uncertainty will be eliminated if future workers in the field of refractometry include the refractive indices in their tables of concentration and scale readings.

In view of the facts presented it is recommended that this committee be continued in order that it may lend its influence toward a successful conclusion of the question and that the Association may be kept properly informed.

R. T. BALCH, *Chairman*
H. C. GORE
C. F. SNYDER

Approved.

REPORT OF COMMITTEE TO CONFER WITH AMERICAN PUBLIC HEALTH ASSOCIATION ON STANDARD METHODS OF MILK ANALYSIS

During the year the committee prepared a revision of Part II of the Standard Methods of Milk Analysis of the American Public Health Association. The manuscript of the revision was submitted to the secretary on methods, Dr. John F. Norton, on July 26th of this year with the recommendation that the methods described be adopted by that Association.

The revision included methods for the analysis of milk, cream and ice cream as now appear in the *Methods of Analysis* of this Association, revision of 1930. Permission for quoting these methods was granted by the Executive Committee. The revision also includes methods for the determination of active or available chlorine in certain preparations used for sterilizing milk equipment and utensils. These methods were included by request of the American Public Health Association and are quoted from Wiley's Principles and Practice of Agricultural Analysis, 3rd Edition, and from U. S. Pharmacopoeia X, with permission of the Executive Committee of the A.O.A.C. and of the Board of Trustees of the U. S. Pharmacopoeial Convention, respectively.

The Committee gratefully acknowledges the cooperation of Miss Lapp and of Dr. LeClerc, who carefully reviewed the manuscript.

E. M. BAILEY, *Chairman*
F. C. BLANCK
G. G. FRARY

Approved.

REPORT OF REPRESENTATIVES ON THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE

In the year 1932 the Crop Protection Institute administered the following projects:

1. *New Copper Fungicides.*

A search for new and better copper compounds adapted for control of plant diseases and intended to eliminate the plant injury often caused by present compounds was carried out in cooperation with the Delaware Experiment Station. Work still in progress has resulted in the discovery of some new materials of definite promise.

2. *Copper Salts in Relation to Plant Nutrition and Plant Stimulation.*

Carried on in cooperation with the Delaware Experiment Station. The work is still in progress and is yielding results that appear to shed a new light on the relation of copper to plant growth.

3. *Oil Sprays for Application to Plants.*

Conducted in cooperation with the Illinois Experiment Station. Two materials now on the market were developed in the course of this investigation, and additional combinations and materials are in process of development. The work is still in progress.

4. *Plant Introduction and Improvement.*

The project was carried on with the cooperation of the New Jersey Experiment Station. The work is still in progress and is yielding information of material promise.

5. *Flotation Sulfur.*

A project conducted with the cooperation of the Illinois Experiment Station. The colloidal or flotation sulfur developed in the course of this project is now in extensive use by fruit growers. The work is being continued with the intent of rounding out the optimum use of the material that has been developed.

6. *Pyrethrum Extract and Pyrethrum Dust.*

This project is maintained in cooperation with the Massachusetts Experiment Station. Extensive and important data have been accumulated as to the best methods of extracting pyrethrum flowers for the manufacture of sprays intended for application to plants, and the best methods of impregnating inert dusts with the active principle of pyrethrum flowers.

7. *The Use of Industrial Adhesive Tape for the Protection of Grafts.*

Conducted in cooperation with the Wisconsin Experiment Station. The project has developed a practical method of using industrial adhesive tape in such way as to reduce losses from crown gall.

8. *Relation of Oil Sprays to Control of the Fruit Moth.*

This investigation was conducted in cooperation with the Delaware Experiment Station and brought to a close early in the present year.

9. *Oil Sprays for the Control of the Codling Moth.*

This project was carried on with the cooperation of the Washington Experiment Station. Work is still in progress.

10. *New Contact Insecticides.*

A preliminary project carried on in cooperation with the Iowa Experiment Station. New and promising materials have been developed and are in process of preparation for commercial use. The work is still in progress.

11. *Iodine Salts as Fungicides.*

A part-time project carried on in cooperation with the New Jersey Experiment Station and brought to a close at the end of the present calendar year.

12. *New Contact Insecticides.*

A thorough search of the possibilities of certain groups of chemicals. This project is conducted in cooperation with the Ohio Experiment Station. Promising results have been secured.

13. *Use of Carbon Dioxide in Connection with Fumigation and in Connection with the Application of Spray Materials.*

A thoroughly organized project carried on in cooperation with the Iowa Experiment Station. Extensive data have been secured as to the practical utilization of carbon dioxide in connection with toxic gases that otherwise would be explosive. Other phases of this study are in progress.

14. *Fungicides in Combination with Oil Sprays.*

A project carried on in cooperation with the New Jersey Experiment Station. New fungicides compatible with oil sprays have been developed, one or more of which offers definite promise.

15. *New Contact Insecticides Combined with New Fungicides.*

A thorough search of an extensive group of organic compounds which have not hitherto been explored. This investigation has been carried on in cooperation with the New Hampshire and the Delaware Experiment Stations. Several new materials have been discovered, one or more of which is expected to be developed commercially at an early date. The work is still in progress.

16. *Development of New Insecticides from Certain Chlorinated Compounds.*

A preliminary project carried on in cooperation with the New Hampshire Experiment Station and with the Ohio State University. One new compound devised is giving excellent and important results.

Most of these projects involve more or less chemical research and some of them concern the development of new uses of chemicals and chemical compounds. Many of these projects are of particular interest to insecticide control laboratories.

<div align="right">H. J. PATTERSON
W. H. MacINTIRE</div>

Approved.

REPORT OF SECRETARY-TREASURER

No resignations of referees were received during the year. The Referee on Sugar and Sugar Products, R. J. Balch, has just sent in his resignation owing to the fact that his work takes him into the field and away from Washington each year at the time of the annual meeting. A few of the associate referees were compelled by pressure of official duties to discontinue their work at the beginning of the year.

It is with regret that I have to announce the passing on during the year of the following honored active and former members:

E. H. Jenkins, long connected with the Connecticut Agricultural Experiment Station; Clifford Richardson, one of the charter members of the Association; Richard H. Gaines, a charter member of the Association; A. S. Mitchell, for many years an active member; E. L. P. Treuthardt, a member of the Food and Drug Administration; G. B. Taylor, a former member of the Bureau of Animal Industry; W. C. Holmes, identified with the Color and Farm Waste Division of the Bureau of Chemistry and Soils; and Sam Wiley, a regular attendant for many years. The Committee on Necrology will present a more extended record of these members.

Most of you have received reprints of the lecture delivered last year by Dr. H. C. Sherman. I have had many commendatory letters about it. It was decided to have a similar lecture delivered each year by some person outstanding in one of the fields of work covered by this Association. Dr. F. E. Denny of the Boyce Thompson Institute was selected to deliver the lecture this year and those of you who heard his splendid address will agree that we are setting a high standard for these memorial addresses. We are compiling a list of persons to be invited to address future meetings and I shall welcome any suggestions which you may have.

Many expressions of approval have also been received during the year in regard to the extension of the plan to divide the meetings into sections. The opinion is quite general that more interesting sessions are possible under this system.

No attempt was made this year to get reduced railroad fares under the certificate plan. It was considered that financial conditions would keep many away, and last year with a full attendance we could not get sufficient certificates. It was also hoped that with the frequent special rates

offered by practically all railroads our members could secure reductions in fares without the complication of the certificate plan. In this regard, however, it has been brought to my attention that this meeting should be held either jointly with the Association of Agricultural College Presidents and Station Directors or with the Society of Agronomists. The latter organization usually meets in Washington, and this year it meets on November 12th. If we were to meet at the same time, or just prior to or following their meeting, we could probably get reduced railroad rates. There are advantages and disadvantages. If anyone has any strong convictions on the matter, I shall be glad to hear about it.

During the year, following instruction of the Executive Committee, the Association was incorporated. I do not think it is necessary to read the articles of incorporation. This action was thought to be necessary, particularly by the treasurer, because the burden of this Association is now large. The original incorporators were W. W. Skinner, C. A. Browne, Marian E. Lapp, G. L. Bidwell, B. G. Hartmann, W. S. Frisbie, and F. C. Blanck. It was necessary that a majority of the original incorporators be residents of the District of Columbia. At the meeting held on Sunday evening the Executive Committee decided that the Board of Directors each year shall be composed of the officers and the Executive Committee. It probably would be wise to offer a motion at this time that the action of the Executive Committee recommending to the Association that the officers and Executive Committee form the Board of Directors be approved. (It was moved, seconded, and passed.)

The customary informal dinner was held Tuesday evening at the Cosmos Club. Dr. Browne gave a delightful talk, accompanied by pictures, on his visits to some of the leading agricultural experiment stations abroad. It seemed to be the general opinion that these talks should be continued until Dr. Browne had exhausted his supply of most excellent material.

The financial statement has been divided into three parts as was done last year. The operating account shows a balance of $328.38; $500.00 was withdrawn and deposited with the loan association in order to get interest on this money. The items for books given in the tabulated figures show the service the Association is able to give its members by getting a discount of 20–25 per cent from all book dealers. The secretary's office is glad to render this service.

The burden of auditing the accounts of the Association is now so large that it should be done by an accredited auditor. That is for my own protection as treasurer and also for the protection of the Association. I call to your attention the fact that in 1921 this Association was not bankrupt, but in debt for about $2,000 and had a lawsuit over its head. Those matters have been straightened out, and it is with much gratification that I report to you the following receipts and expenditures from October 1, 1931, to October 1, 1932, and the splendid cash balance of $8,371.50.

PUBLICATIONS
RECEIPTS
Methods of Analysis

Number	Price each	
25	$5.50	$137.50
776	5.00	3,880.00
3	4.50	13.50
157	4.40	690.80
543	4.00	2,172.00
11	3.92	43.12
1	3.00	3.00
1	.67	.67

Total...$6,940.59

Journal

Number	Price each	
45	$5.50	$247.50
350	5.00	1,750.00
26	4.50	117.00
90	4.40	396.00
282	4.00	1,128.00
15	1.50	22.50
7	1.25	8.75
2	1.10	2.20
3	1.00	3.00
	.85	.85

	$3,675.80	
Plus gain on exchange	4.90	

Total..$3,680.70
Minus charge for exchange..   20.39
Minus tax on checks......   .30
Minus redeposited checks .   75.90
Minus returned checks...   9.00   105.59

$3,575.11

Wiley's Principles and Practice of Agricultural Analysis

Number	Price each	
3	$10.00	$30.00
19	7.00	133.00
1	6.67	6.67

Total...$169.67

Advertisements

Number	Price each	
11	$25.00	$275.00
5	15.00	75.00

Total $350.00

Reprints

University of Tennessee, Knoxville, Tenn.............	$9.15
American Pharm. Mfrs. Assoc. N.Y.C................	5.48
Wm. F. Kunke, Chicago, Ill........................	5.55
F. Leslie Hart, Washington, D.C....................	4.16
H. A. Schuette, Madison, Wis......................	4.09
A. L. Prince, Trenton, N. J........................	5.86
E. Runkel, Chicago, Ill...........................	2.68
J. Davidson, Washington, D.C.	4.80
E. P. Clark, Washington, D.C......................	2.79
M. Phillips, Washington, D.C......................	2.85
C. A. Browne, Washington, D.C....................	2.85
Purdue University, Lafayette, Ind..................	7.60
H. C. Sherman, N. Y. C...........................	9.00
K. D. Jacob, Washington, D.C.....................	4.17
Research Corporation, N. Y. City................. .	14.95
G. Pitman, Oakland, Calif.........................	2.00
State of Minnesota, St. Paul, Minn..	5.15
R. I. Experiment Station, Providence, R.I........	4.00
Macdonald College, Quebec, Canada.....	7.97
Total.................................. ....$105.10	

Miscellaneous

Montgomery Mutual Bldg. and Loan Assoc...........	$1,000.00
Dr. James, book...............................	32.38
Dr. Clark, book.......	5.31
Dr. Haller, book..	2.92
Dr. Haller, book.................................	4.94
Dr. Phillips, book..	4.94
Interest on checks.......	.16
Total..$1,050.65	

Total for Methods, Journals, Wileys, Ads, Reprints and Miscellaneous..$12,191.12
Cash in bank, October 1st, 1931................................ 5,633.63

Total..$17,824.75

DISBURSEMENTS

1931			Amount	Check
Oct.	5	Columbia Specialty Paper Box Co...............	$10.00	384
	8	M. A. Bates, office expenses....................	50.00	385
	9	Columbia Specialty Paper Box Co., balance.......	12.50	386
	14	J. J. Betton, premium on bond, M. A. Bates.......	2.50	387
	14	M. A. Bates, stamps for mailing Methods of Analysis.	65.00	388
	15	Geo. Banta Publishing Co., reprints, May Journal..	22.06	389
	15	Geo. Banta Publishing Co., Wiley Memorial.......	17.37	390
	15	Ace Letter Service, circular letters, Methods.......	44.75	391
	26	Refund to Association Acct., dues Tenn. Agri. Expt. Station....................................	5.00	392
	26	P. Blakiston's Sons, books for Dr. James..........	32.38	393

Nov.	6	Chemical Publishing Co.,Wiley Book............	333.33	394
	21	Geo. Banta Publishing Co., November Journal.....	1,096.04	395
	21	Geo. Banta Publishing Co., insurance and storage of books.....................................	33.00	396
	21	H. F. Warneson & Co., binding two vols. Journal...	4.00	397
	24	M. A. Bates, stamps...........................	65.00	398
	24	Geo. Banta Publishing Co., Methods of Analysis, 1930...............	4,000.00	399
Dec.	2	Joseph Cohen, affidavits......................	10.75	400
	12	Geo. Banta Publishing Co., balance on bill for 1930 ed., Methods of Analysis.................. ..	952.02	401
	15	Frances S. Beatty, salary...........	10.00	402
	15	W. F. Roberts Co., labels, Methods.	18.00	403
	17	M. A. Bates, stamps..........	65.00	404
	21	Frances S. Beatty, salary..............	10.00	405
	21	Postmaster, quarter ending 1/1/32.....	2.00	406
1932				
Jan.	4	Frances S. Beatty, salary........	15.00	407
	4	W. F. Roberts Co., letterheads.	6.00	408
	8	Frances S. Beatty, salary.....................	10.00	409
	14	Geo. Banta Publishing Co., reprints, Vol. XIV, No. 4	45.92	410
	14	Univ. of Kentucky, refund on subscription... . .	1.00	411
	14	M. A. Bates, office expenses............ . . .	65.00	412
	20	Robert C. Jones & Co., Treasury Bond	923.91	413
	21	Frances S. Beatty, salary, Jan. 4–18..	30.00	414
Feb.	1	Frances S. Beatty, salary, Jan. 19–31......... .	30.00	415
	1	Refund to Assn. Acct., dues, Utah Expt. Station...	5.00	416
	1	Refund to Assn. Acct., Kentucky Expt. Station dues	5.00	417
	4	John Wiley & Sons, books for Dr. Haller.. .	2.92	418
	4	Blakiston's Son & Co., book for Dr. Clark... . .	5.31	419
	4	Geo. Banta Publishing Co., storage of Journals ...	3.00	420
	11	Turner Sub. Agency, refund on subscription..... .	4.40	421
	11	Merchants Transfer & Storage Co., books from printer...............	2.49	422
	15	Frances S. Beatty, salary.................. .	30.00	423
	17	R. S. Hollingshead, Journals, Vols. 1 and 2 .	8.00	424
	19	M. A. Bates, office expenses...	50.00	425
	29	Frances S. Beatty, salary...............	30.00	426
Mar.	3	Geo. P. Killian, boxes for mailing Methods.....	29.99	427
	7	Geo. Banta Publishing Co., freight on Methods .	41.98	428
	10	Geo. Banta Publishing Co., February Journal. .	879.85	429
	14	Frances S. Beatty, salary....	30.00	430
	14	M. A. Bates, office expenses........	65.00	431
	28	Postmaster, quarter ending April 1....	2.00	432
	29	Frances S. Beatty, salary..................	30.00	433
	30	Harris & Ewing, photograph, Dr. Sherman .	3.00	434
April	1	E. O. Huebner, refund on subscription . .	1.00	435
	1	Geo. P. Killian Co., balance on bill..............	2.50	436
	7	Geo. Banta Publishing Co., reprints, February Journal.................................. ...	87.98	437
	7	Ace Letter Service, invoice forms................	11.75	438
	19	Frances S. Beatty, salary................	30.00	439
	22	M. A. Bates, cash for stamps...................	50.00	440

May	2	Geo. Banta Publishing Co., reprints, May Journal..	40.66	441
	2	Geo. Banta Publishing Co., storage of books.......	3.00	442
	3	Frances S. Beatty, salary.......................	30.00	443
	4	M. A. Bates, 1½¢ stamps.....................	5.00	444
	12	W. F. Roberts Co., letter heads	15.00	445
	23	Frances S. Beatty, salary.......................	30.00	446
	23	John Wiley & Sons, books for Dr. Haller..........	9.88	447
	31	Frances S. Beatty, salary.......................	30.00	448
June	3	H. F. Warneson & Co., binding 11 vols. Journal....	22.00	449
	3	J. J. Betton, premium on bond, M. E. Lapp.......	5.00	450
	3	M. A. Bates, office expenses....................	50.00	451
	7	Refund to Assn. Acct., Georgia and Iowa..........	10.00	451b
	14	Frances S. Beatty, salary.......................	30.00	452
	21	Geo. Banta Publishing Co., May Journal..........	1,047.32	453
	21	Postmaster, quarter ending June 30..............	2.00	454
	23	Refund to Assn. Acct., Kansas State College	5.00	455
	28	Frances S. Beatty, salary.......................	30.00	456
July	5	Geo. Banta Publishing Co., storage of Journals.....	3.00	457
	5	Refund to Assn. Acct., dues, Vermilion, N.D.	5.00	458
	6	John Wiley & Sons, book for Dr. Haller...	1.50	459
	12	Frances S. Beatty, salary.......................	30.00	460
	12	M. A. Bates, cash for stamps...................	50.00	461
	26	Geo. Banta Publishing Co., reprints, May Journal...	89.94	462
	26	Frances S. Beatty, salary.......................	30.00	463
Aug.	3	Joseph Cohen, affidavits.......................	10.50	464
	13	Franklin Square Agency, refund on subscription....	4.00	465
	15	John Wiley & Sons, book for Mr. Swenson........	2.48	466
Sept.	19	M. A. Bates, office expenses.....................	50.00	467
	19	Maruzen Co., Japan, refund on cancelled order...	2.20	468
	19	Geo. Banta Publishing Co., August Journal........	942.92	469
	19	Ace Letter, Service, letters re. Wiley Memorial.. .	3.45	470
	20	Postmaster, quarter ending October 1st........ ..	2.00	471

Total checks...........................$11,990.55
Cash in bank October 1st, 1932... .... 5,834.20

$17,824.75

OPERATING ACCOUNT
RECEIPTS
1931

Oct.	1	Bank balance...............................	$694.90
		1931 dues from institutional members, 63 at $5.00.	315.00
		Coupon from bond.........................	16.87
		Miscellaneous.............................	5.07

$1,031.84

DISBURSEMENTS
1931   Check No.

Oct.	29	Marian E. Lapp, expenses, 1930 meeting.......	$40.00	88
Nov.	7	Expenses, Executive Committee..............	15.00	89
Nov.	24	H. C. Sherman, attendance at meeting........	25.29	90
Dec.	2	W. F. Roberts Co., programs for meeting.......	63.50	91

1932
Feb. 19   Investment, Montgomery Mutual Bldg. and Loan
                Assoc................................   500.00   92
Feb. 19   Safe deposit box.......... ....   2.50   93
July 5   Attorney, services relative to incorporation of
                Assoc............. ....   27.15   94
Sept. 19   M. A. Bates, stamps for programs 30.00   95
           Tax on checks. 02

                                                           703.46
Oct. 1   Cash in bank 328.38

            Total $1,031.84

SUMMARIZED STATEMENT
ASSETS

Publications account balance $328.38
Operating account balance 5,834.20
Savings account (Montgomery Mutual Bldg. & Loan Assoc.). 1,208.92
Treasury gold certificate 1,000.00
13,396 (approximately) copies of *Journal* appraised   . ..10,029.95
3000 (approximately) *Methods of Analysis*. 12,000.00

            Total $30,401.45

LIABILITIES—*None*

Approved.   W. W. SKINNER

REPORT OF COMMITTEE TO COOPERATE WITH OTHER COMMITTEES ON FOOD DEFINITIONS

This committee respectfully submits the following report of its proceedings since the last meeting of the Association.

In harmony with the program of governmental retrenchment and economy, but one meeting of the committee was held during the year, this, the 42nd, occurring the week of April 11.

On April 13 the committee gave a public hearing on strained tomato products. Following, tentative adoption was given to a definition and standard for the product known as heavy tomato puree. Definitions and standards finally adopted for other tomato products are as follows:

Tomato puree, * *Tomato pulp*, is the product resulting from the concentration of the screened or strained fleshy and liquid portions of ripe tomatoes, except those portions from the skin and core trimmings; with or without the addition of salt. The product contains not less than 8.37 per cent of tomato solids.

* "Tomato puree" should not be confused with "puree from trimmings," a term used to denote a product, commonly unconcentrated, sometimes added in the canning of tomatoes.

Tomato paste, Salsa di pomodoro, "Salsa," is the product resulting from the concentration of the screened or strained fleshy and liquid portions of ripe tomatoes,

except those portions from skin and core trimmings; with or without the addition of salt, and with or without the addition of basil. The product contains not less than 22 per cent of tomato solids.

Heavy tomato paste, "Concentrato" is tomato paste containing not less than 33 per cent of tomato solids.

The existing definition for canned tomato juice was amended to read as follows:

Canned tomato juice is the unconcentrated, pasteurized product, consisting of the liquid, with a substantial portion of the pulp, expressed from ripe tomatoes with or without the application of heat; and with or without the addition of salt.

The moisture content of dried fruits was considered at some length, and tentative definitions and standards as a basis for further consideration were formulated for those varieties commonly subjected to sulfuring, viz., apricots, peaches and prunes. A tentative definition and standard for apple butter was also developed. The committee was assisted in the formulation of these proposals through the presentation of testimony by specialists in the Administration and in the trade.

The subject of corn meal was extensively discussed and some testimony was received as a preliminary to the ultimate adoption of definitions for the various forms of these cereal products. This matter is pending until the committee obtains further data.

The committee noted receipt of a request for definitions for glacé fruits, which matter was considered and action thereon deferred. Similar action was taken respecting a request for a definition for whole wheat crackers.

Because of the rather general interest which attaches, it may be worth while to present here what may be termed the code of committee procedure, as adopted in amended form at this meeting. The text of these rules is as follows:

The subject matter for the consideration of the committee should include all material coming within the scope of the Federal food and drugs act or the food laws of the various States in so far as these acts or those laws relate to articles of food for human consumption.

All information and data which may be used as the basis for definitions and standards shall be discussed and reviewed by the committee as a whole. The secretary shall, so far as possible, provide the members with copies of all information bearing upon the topics under consideration, including correspondence which has been submitted by the trade or other interested parties. Information secured by individual members of the committee shall be communicated to the secretary for distribution to committee members.

Public notices of all hearings shall be made at least two weeks prior to the date set for the hearing. These notices shall be sent, so far as possible, to all those interested: trade papers, secretaries of the associations interested in the hearing, State food officials, State home economics departments, and administrative units of the Food and Drug Administration.

No member of the committee shall express orally or in writing any opinion, or make any statement, which tends to represent a committee opinion, unless the committee has already definitely expressed itself. A like discretion shall be observed to refrain from revealing individual differences of opinion within the committee.

No member shall discuss any affirmative action taken by the committee prior to public notice through the Food and Drug Administration.

Members of the committee may make reports of the progress of the committee's work to the respective organizations which they represent; discuss scope and general methods of procedure governing the committee's work, and may answer inquiries as to what topics are under consideration by the committee.

Once more the committee is called upon to record the passing of one of its members. The death of Andrew S. Mitchell occurred on August 18 of this year following a brave fight against an insidious malady. Long a member of this Association, Mr. Mitchell for many years had served the Standards Committee faithfully and well as its secretary. In the gathering of data, the transmitting of correspondence, the preparation for meetings and in various other ways he had, in recent years, devoted a very large share of his time to these duties. Ad interim, Mr. Mitchell was, in fact, "the Standards Committee." Intensely interested in its work, his was a labor of love. Valuable as were his help and guidance to the committee, its members feel with especial keenness the loss of a good friend and cheerful comrade.

<div style="text-align: right">

C. D. HOWARD, *Chairman*

G. G. FRARY

E. M. BAILEY

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Approved.

No report was made by the Committee on Sampling.

No report was made by the Committee on Bibliography.

REPORT OF AUDITING COMMITTEE

The Auditing Committee examined the accounts of the Secretary-Treasurer of the Association of Official Agricultural Chemists and the accounts of the same official for *The Journal* of the Association, and for *Methods of Analysis* from October 1, 1931, to October 1, 1932, and found the same correct.

<div style="text-align: right">

B. G. HARTMANN

B. E. BROWN

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Approved.

REPORT OF COMMITTEE ON NECROLOGY

Since the date of the last meeting the Association of Official Agricultural Chemists has again suffered several severe losses from the deaths of prominent members who in former years have contributed to the advancement of its work. The list includes three charter members of our association, and four former referees and collaborators. A brief mention of their names and of their contributions to the work of the Association is attached herewith.

(1) *E. H. Jenkins* (1850–1931), one of the founders of our Association, member of its first Executive Committee upon Food Standards and for many years an active and faithful promoter of all its activities. A full account of his long and fruitful career as director of the Connecticut Agricultural Experiment Station was published in the recent August number of *The Journal* of the Association.

(2) *Clifford Richardson* (1856–1932), one of the founders of our Association, its secretary-treasurer from 1885 to 1890, editor of its proceedings for the third, fourth and fifth annual meetings, and a member of its first committee upon potash. He was a loyal and most efficient supporter of the early work of the Association. A sketch of his professional career as a chemist of the U. S. Department of Agriculture and as a chemical engineer and consultant is published in the recent November issue of *The Journal*.

(3) R. H. Gaines (1862–1932), one of the founders of our Association, and a frequent attendant at the meetings during the first fifteen years. He was born at Mossingford, Va., on February 10, 1862, and after graduation from the University of Virginia became later chief chemist of the Virginia State Laboratory at Richmond, a position which he retained until 1899. It was during this period that he became strongly interested in the work of our Association. From 1906 until the time of his death Mr. Gaines was chief chemist of the Water Supply Board of New York City. He was a chemist of outstanding ability, and his genial personality endeared him to a host of friends.

(4) *A. S. Mitchell* (1864–1932), for thirty-five years a member and frequent attendant at the meetings of our Association, of which he was a zealous supporter both by his various duties as referee and by his friendly spirit of cooperation. His faithful service as member and secretary of the Food Standards Committee will long be remembered by those who were associated with him in this work. A sketch of his career will shortly be published in our *Journal*.

(5) *E. L. P. Treuthardt* (1885–1932), an associate chemist of the Boston Station of the Food and Drug Administration and for twenty years an active participator in the work of our Association. His first collaborative work upon flavoring extracts was done in 1911. His exhaustive re-

ports as Associate Referee on Heavy Metals in Foods were published in the proceedings of the Association for the years 1914 and 1915. His last report as Associate Referee on Dried Milk was presented before the Association in 1931. His passing removes one of the most faithful and experienced analysts of our society.

(6) *G. B. Taylor* (1878–1931), formerly a chemist with the State Board of Health of New Orleans, later a chemist with the Bureau of Animal Industry and for the past twelve years the chief of the laboratories of the Chestnut Farms Dairy Corporation of Washington, D. C. Between 1912 and 1917 he participated in the work of the Association as a collaborator in the work upon alkaloids and dairy products. He was a man of most pleasing personality, and his unfortunate death in an airplane accident at Camden, N. J., removed one of America's most competent executives in the field of applied agricultural chemistry.

(7) *W. C. Holmes* (1884–1932), senior chemist of the Color and Farm Waste Division of the Bureau of Chemistry and Soils and an occasional participator in the work of our Association. His reports upon the "Iodometric Evaluation of Methylene Blue" and the "Spectrophotometric Detection of Boron" were published in Vol. X of *The Journal* of the Association. As consulting editor of "Stain Technology" and the American Chemical Society representative on the Commission for Standardization of Biological Stains he was recognized as the leading expert of the United States in this special field. He contributed greatly to the development of new and better biological stains, and his sudden untimely death removed one of the most gifted scientists in the Government Service.

These departed members have inspired us all by their examples of loyalty, public service, and unselfish devotion to the work in which we are all engaged, and this brief memorial is only a faint expression of the appreciation in which their names are held. In addition to these names I might mention also the recent passing of a chemist who at one time was a frequent attendant at our meeting, although he never took an active part, that of the late Dr. Langworthy. I move you, Mr. President, that we all rise as a token of respect to their memory.

C. A. BROWNE, *Chairman*

Approved.

REPORT OF NOMINATING COMMITTEE

The committee respectfully submits the following report:

President: J. W. Kellogg, Harrisburg, Pa.
Vice-President: R. Harcourt, Guelph, Canada.
Secretary-Treasurer: W. W. Skinner, Bureau of Chemistry and Soils, Washington, D. C.

Additional Members of the Executive Committee:

F. C. Blanck, Washington, D. C.
H. H. Hanson, Dover, Del.
C. C. McDonnell, Washington, D. C.
Post Officio, A. E. Paul, Chicago, Ill.

R. N. BRACKETT
J. S. McHARGUE
R. C. ROARK

A unanimous vote was cast for the officers nominated.

REPORT OF COMMITTEE ON RESOLUTIONS

Notwithstanding the depression this, the 48th annual meeting of the A.O.A.C., has been one of the most successful ever held. The flourishing financial condition of this Association as shown by the secretary's report, and the continuation of the plan to hold sectional meetings which have again proved so successful, both in attendance and in the maintenance of interest throughout the sessions, are evidences of wise and sound leadership. It is therefore eminently fitting at this time that we again express our appreciation to those who have helped to preserve the ideals and traditions of this Association and to make this convention stand out as one of the best in its history. Be it therefore

(1) Resolved: That this Association reaffirm the tributes that have been paid by the Committee on Necrology to the following members who since our last meeting have passed on to another sphere: E. H. Jenkins, Clifford Richardson, E. L. P. Treuthardt, A. S. Mitchell, E. B. Taylor, W. C. Holmes and C. F. Langworthy, and that a copy of the respective tributes to the memory of our former co-workers, when published in our *Journal,* be sent to the family of each deceased member.

(2) Resolved: That this Association express its thanks to Dr. A. F. Woods, Director of Scientific Research, for his cordial greetings and his able and vigorous message.

(3) Resolved: That this Association extend to Dr. F. E. Denny, Boyce Thompson Institute for Plant Research, its most sincere thanks for his exceedingly interesting and valuable Wiley Memorial Address on the effect of chemical stimulants on dormant plants.

(4) Resolved: That this Association express its appreciation to Mr. A. E. Paul, retiring president, for the courteous and painstaking manner in which he has discharged the duties of his office, and for his inspiring

presidential address upon the necessity and value of conservatism as a means to true progress.

(5) *Resolved:* That this Association acknowledge once more its indebtedness to its Secretary-Treasurer, Dr. W. W. Skinner, for the highly efficient manner in which he has continued to serve it.

(6) *Resolved:* That we convey our thanks to Mr. W. S. Frisbie and his co-workers of the Board of Editors of our *Journal,* for the continued high financial and scientific standing of this publication.

(7) *Resolved:* That this Association again extend its sincere appreciation to Miss Marian E. Lapp and her co-workers for their efficiency in the issuance of our *Journal* and other publications, and for the gracious and efficient manner in which she and her co-workers have assisted the members of this Association at this meeting.

(8) *Resolved:* That the plan of holding a symposium, which has proved so successful this year, be approved and that arrangements be made to hold a similar symposium at our next meeting.

(9) *Resolved:* That this Association acknowledge with sincere appreciation the cordial and friendly message of the A.A.C.C.

(10) *Resolved:* That this Association extend to the management of the Raleigh Hotel a vote of thanks for the accommodating manner with which it has served us and for the many courtesies which have been extended to our members and friends at this meeting.

> J. A. LeClerc, *Chairman*
> H. R. Kraybill
> C. C. McDonnell

Approved.

A. E. Paul: I desire at this time to thank this Association and its members for the privilege of having served as its president. It has been a pleasure and an inspiration to me. Since the meeting is now closed it remains for us to look progressively forward to another year of accomplishment, another annual convention. You have chosen as your leader a man preeminently qualified for this leadership, a man whom we all know and like, a man whom we shall all assist and support in every possible manner. It is with pleasure that I now hand to him this gavel and present to you our president, Dr. J. W. Kellogg.

J. W. Kellogg: Mr. Chairman, and members of the A.O.A.C., I deeply appreciate this honor which has come to me after having served twenty-five years in Pennsylvania, which really comes on my 25th birthday, so to speak. I look forward to our next coming together a year from now, here in Washington, when I take the gavel which has been presented to me and which I understand my friend Dr. Brackett wielded in 1916. I

think he went out in the forest somewhere and chopped down a tree on the estate of John C. Calhoun and made this. In 1917, I think it was, on his 16th birthday. I was a little bit fearful that Dr. Brackett would not nominate me. I understand there are several other well-known men (I don't mean to infer that I'm so well known) who were running for president this time and he was interested in one of the other two. Any other business to come before the conference at this time? If not, movement to adjourn is in order.

CONTRIBUTED PAPERS

RÉSUMÉ OF A METHOD FOR THE DETERMINATION OF FLUORINE[1]

By O. B. WINTER and LILLIAN BUTLER (Michigan Agricultural Experiment Station, East Lansing, Mich.)

The difficulty in doing experimental or control work involving fluorine compounds has been due to the fact that there was no satisfactory method for accurately determining the fluorine content of the materials under consideration. A new method for the determination of this element has been developed by Willard and Winter.[2] The method is based on the following principles: (1) Fluoride solutions may be titrated with standard thorium nitrate and the use of a zirconium-alizarine mixture as the indicator, and (2) fluorine may be separated from interfering elements by volatilizing it as hydrofluosilicic acid. The general procedures for carrying out these two principles, quoting from the original article,[2] show that the entire method is comparatively short and that the apparatus is simple and easy to manipulate.

Titration of fluoride solutions when no interfering elements are present.—Transfer an aliquot of the solution to be analyzed to a small tall-form beaker, and add water to make a volume of approximately 20 cc. and 3 drops of the zirconium-alizarine mixture. If necessary, add just enough dilute hydrochloric acid to destroy the color. Add an equal volume of neutral ethyl alcohol and titrate over a white surface in good light with the standard thorium nitrate to a faint permanent reappearance of color.

Volatilization of fluorine as hydrofluosilicic acid.—Place the sample in a small distillation flask, and add a few glass beads or pieces of porous plate, 5 cc. of 60 per cent perchloric acid, and sufficient water to cause the solution to boil at 110° C. or less. Place the flask on an asbestos mat with an opening sufficiently large so that about one-third of the flask will be exposed to the flame, and close with a two-holed rubber stopper through which passes a thermometer and a capillary tube, both of which extend down into the liquid. Connect a dropping funnel with the capillary tube so that water may be added during the process of distillation, fill with water, and connect the flask with a water condenser. (The distillate may be collected in an open container.) Distil until the boiling point of the solution reaches 135° C. and hold at approximately that temperature by allowing water to run into the flask from a dropping funnel until all the fluorine has been volatilized. This is usually accomplished by distilling over 50–75 cc. Add 6 drops of the zirconium-alizarine mixture and dilute sodium hydroxide drop by drop until the color of the indicator appears. Add an equal volume of ethyl alcohol and then dilute acid until the color of the indicator just disappears (at this point it is advisable to cause the color to appear and disappear two or three times to be sure to have the solution only very slightly acid). Titrate with thorium nitrate as previously directed.

[1] Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., November, 1932.
[2] Ind. Eng. Chem. Anal. Ed. 5, 7 (1933).

The method was found to be accurate for the determination of fluorine in all the materials analyzed. However, volatilization of fluorine is retarded by the presence of boric acid, gelatinous silica, or aluminum, and when a considerable quantity of any of these elements was present, it was found necessary to modify the procedure.

Following the presentation of the above mentioned paper, some work was done on the determination of fluorine in plant materials. The following procedure was used for this work:

Fluorine in plant materials.—Moisten 5–20 grams of material with saturated lime water, dry, and ignite at dull redness in a muffle. If the sample is not thoroughly ashed, break up the ash if necessary, moisten with water, dry, and reignite.[1] Transfer to the distillation flask, and add water, a few pieces of glass or porous plate, and perchloric acid drop by drop with shaking until the solution becomes acid, using litmus paper as an external indicator. Now add 5–8 cc. of perchloric acid and determine the fluorine as previously described under the volatilization of fluorine as hydrofluosilicic acid.

Several fluorine determinations were made by the above method. These include the analysis of a fluoride, the analyses of the same fluoride when added to plant materials before and after ashing, and the analyses of plant materials and bones. The results are given in the following table.

Fluorine in miscellaneous materials.

MATERIAL	SAMPLE	DISTIL-LATE	FLUORINE	
			PRESENT	FOUND
	gram	*cc.*	*per cent*	*per cent*
Rock Phosphate..............................	0.1	50	3.52	3.49
Rock Phosphate with ash from 20 gm. Rice.......		50		3.50
Rock Phosphate ignited with 20 gm. Rice.........		100		3.38
Rock Phosphate ignited with 5 gm. Alfalfa.......		50		3.32
Rock Phosphate ignited with 5 gm. Alfalfa........		75		3.46
Rock Phosphate ignited with 5 gm. Alfalfa........		75		3.22
Rock Phosphate ignited with 5 gm. Alfalfa.......		75		3.14
Rock Phosphate ignited with 5 gm. Alfalfa........		75		3.32
Rock Phosphate ignited with 2 gm. Alfalfa........		100		3.34
Rock Phosphate ignited with 2 gm. Alfalfa........		50		3.43
Rock Phosphate ignited with 5 gm. Wheat........		75		3.46
Rock Phosphate ignited with 5 gm. Wheat........		75		3.43
Rice..		50		0.00091
Spinach.....................................		125		0.00086
Spinach.....................................		50		0.00063
Corn and Oats...............................		125		0.00085
Corn and Oats...............................		125		0.00104
Bones (Normal Cow).........................		50		0.0158
Bones (Fluorine fed Cow).....................		50		0.020
Bones (Fluorine fed Cow).....................		50		0.022

[1] The organic matter should be well oxidized, or the reaction may become unduly violent when boiling with perchloric acid.

The results given in the table show that when a fluoride was added to the ash from plant material, the fluorine could be recovered quantitatively by the volatilization method, and that when a fluoride was added to plant material and the material burned at dull redness the amount of fluorine recovered varied from 89 to 98 per cent. The results also show that higher recoveries were made from wheat than from alfalfa.

These data indicate that the recovery of fluorine from plant ash needs further study. The temperature of burning, the ratio of calcium hydroxide to sample, the quantity of silica present in the ash, and the use of other materials than calcium hydroxide for neutralizing are factors which may have a bearing on the recovery of the fluorine.

A STUDY OF THE KJELDAHL METHOD

I. MERCURIC OXIDE AS A CATALYST WHEN BLOCK TIN CONDENSERS ARE USED[1]

By R. A. Osborn and Alexander Krasnitz (Bureau of Chemistry and Soils,* U. S. Department of Agriculture, Washington, D. C.)

In the official Kjeldahl-Gunning-Arnold method[2] for the determination of nitrogen, mercuric oxide is recommended as a catalyst. Following digestion of the sample the procedure calls for the precipitation of the mercury as sulfide before distillation of ammonia from alkaline solution. The writers learned recently from private discussions with analytical chemists that the use of block tin condensers during distillation is considered to be a potential source of error when the mercury catalyst is employed as specified in the official method. It was pointed out that mercury is volatilized during the distillation and condenses to form a film or amalgam on the inner surface of the tin, which surface may adsorb or react chemically with ammonia. One laboratory, at least, is reported to have replaced block tin condensers with a good grade of glass to obviate this source of error.

It was, therefore, considered advisable to make a study of this situation with a view to determining to what extent, if any, the inner surfaces of block tin condensers, long in use, retain ammonia. That mercury is volatilized during distillation had been noted by the writers. It occasionally appears in the flask receiving the distillate, at times as a dark colloidal suspension, and again as dark globules which readily coalesce. Upon examination, the inner surfaces of the old block tin condensers being used were found to be covered with deposits of mercury.

In the course of this experimental study, the following reagents and

standard solutions were employed: distilled water, ammonium chloride solution—4.0000 grams of reagent quality salt dissolved in distilled water and diluted to 1000 cc., a 0.2 per cent water solution of sodium alizarin sulfonate indicator, 0.5000 N and 0.0200 N hydrochloric acid and 0.2000 N, 0.0200 N and 10 per cent sodium hydroxide solution.

Table 1 indicates the milligrams of nitrogen obtained by the following procedure: 25 cc. of standard ammonium chloride solution was pipetted into Kjeldahl flasks to which were added 250 cc. of distilled water and 20 cc. of 10 per cent sodium hydroxide solution. Distillations were carried out with seventeen stills containing block tin condensers which had been in use sufficiently long to have acquired a deposit of mercury upon their inner surfaces. An average of 150–200 cc. of distillate was collected in 500 cc. Erlenmeyer flasks containing 5 cc. of 0.5 N hydrochloric acid, 20 to 25 cc. of distilled water, and four drops of the indicator solution. Titration of the excess acid was made with 0.2000 N sodium hydroxide.

TABLE 1.

Distillation of ammonia from 25 cc. of ammonium chloride solution.

STILL NO.	N₂ FOUND	INCREASE OVER THEORETICAL	STILL NO.	N₂ FOUND	INCREASE OVER THEORETICAL
	mg.	*mg.*		*mg.*	*mg.*
1	26.5	0.32	15	26.5	0.32
2	26.3	0.12	16	26.3	0.12
3	26.6	0.42	17	26.5	0.32
5	26.6	0.42	18	26.5	0.32
7	26.5	0.32	19	26.6	0.42
8	26.6	0.42	20	26.3	0.12
9	26.6	0.42	21	26.3	0.12
13	26.3	0.12	22	26.6	0.42
14	26.5	0.32	Av.–17 stills	26.48	0.30

It will be observed from the table that the calculated amount of nitrogen taken for analysis was 26.18 mg., whereas the average weight of nitrogen obtained was 26.48 mg. The average deviation from the average is 0.10 mg., and the maximum deviation, 0.18 mg. It is not possible to conclude from Table 1 that no ammonia was derived from the condensers, but an explanation must be made for the 0.30 mg. of nitrogen increase and its relationship, if any, to the condensers.

Table 2 indicates the results from three series of distillations of distilled water through the block tin condensers. In series A, 250 cc. portions of water were placed in clean Kjeldahl flasks, no alkali was added, and approximately 150 cc. of water was distilled and collected in receiving flasks containing 1 cc. of 0.0200 N hydrochloric acid, 20 cc. of water, and four drops of indicator solution. The excess of acid was titrated with 0.0200 N sodium hydroxide solution.

In series B distillations the condensers were not cooled by a flow of water, and this caused an increase of approximately 85° C. in their temperature. In all other respects this series resembled series A. Distillation of series B was discontinued when the temperature of the solution in the receiving flasks reached the boiling point except in the cases of Nos. 13 and 14, where the receiving flasks were cooled by an ice bath.

In distillations of series C, 20 cc. of 10 per cent sodium hydroxide and a small quantity of finely divided zinc were added to 250 cc. of distilled water, and the distillation was carried out as described in series A. This series simulated the conditions of distillation employed with ammonium chloride solution.

Before the data from Table 2 are discussed, it should be mentioned that 200 cc. portions of distilled water were titrated with 0.0200 N hydrochloric acid and sodium alizarin sulfonate indicator. Duplicate titrations of the ordinary and the freshly boiled distilled water gave identical results, which expressed in terms of nitrogen were equivalent to 0.12 mg. Titration of 300 cc. portions of distilled water required correspondingly greater amounts of 0.0200 N. acid. The agreement among results in Table 2 is much closer than that found in Table 1. This is logical if the volumes

TABLE 2.
Nitrogen obtained from distillation of distilled water through block tin condensers.

STILL NO.	A CONDENSERS COOLED NO ALKALI ADDED TO WATER	B CONDENSERS NOT COOLED NO ALKALI ADDED TO WATER	C CONDENSERS COOLED 10 PER CENT ALKALI ADDED TO WATER
	mg.	*mg.*	*mg.*
1	0.13	0.13	
2	0.17	0.14	0.08
3	0.13	0.10	0.10
5	0.14	0.14	0.11
7	0.15	0.17	0.10
8	0.15	0.14	
13	0.20	0.21*	0.10
14	0.13	0.11*	0.14
15	0.10	0.15	0.08
16	0.21	0.21	0.18
17	0.13	0.15	
18	0.15	0.14	0.14
19	0.14	0.14	0.14
20	0.11	0.18	
22	0.11	0.17	
Av.	0.14	0.15	0.12

* Ice bath around receiving flasks.

and strengths of the standard solutions employed are considered. It will be observed from Table 2 that there are no significant differences between

series A, B, and C. If appreciable quantities of ammonia were held by the block tin condensers, it would be reasonable to expect the results of series B to be higher than those of series A. The results of series A, B and C when considered with those obtained by the titration of ordinary and freshly boiled distilled water, indicate the absence of significant quantities of either free or combined ammonia. It would appear, therefore, that the "alkalinity" obtained is largely due to the acid requirements of 200 to 250 cc. of this water in shifting the pH to that at which sodium alizarin sulfonate responds.

A STUDY OF THE KJELDAHL METHOD[1]

II. COMPARISON OF SELENIUM WITH MERCURY AND WITH COPPER CATALYSTS

By R. A. OSBORN and ALEXANDER KRASNITZ (Bureau of Chemistry and Soils,* U. S. Department of Agriculture, Washington, D. C.)

Lauro[2] first suggested the use of selenium and its oxychloride as a catalyst in the Kjeldahl-Gunning-Arnold[3] method for the determination of nitrogen, and Sandstedt,[4] Rich,[5] and Messman[6] have devoted additional study to its use in the analysis of cereal products.

Data on the comparative efficiency of selenium and other catalysts, however, are incomplete and at variance, and therefore additional study was considered to be desirable.

A number of duplicate analyses were made with a gluten flour and with an ordinary flour in which the length of the digestion period was varied from 30 to 150 minutes. One gram samples of these flours were analyzed[3] with additions of 25 cc. of sulfuric acid, 11 grams of anhydrous sodium sulfate, and with either 0.7 gram of mercuric oxide, 0.1 gram of selenium (precipitated), 0.2 gram of selenium oxychloride, or 1.0 gram of copper sulfate ($CuSO_4 \cdot 5H_2O$) as catalyst. The flasks and their contents were placed on calibrated 400 Watt electric heaters and rotated from time to time during the digestion.

Table 1, A and B, summarizes the data obtained. It is apparent that all digestions must be continued beyond the time required for clearing in order to obtain correct results. With the gluten flour a digestion time of 60–75 minutes is necessary, while with the ordinary flour 40–45 minutes appears to be sufficient. With both flour samples a digestion time up to 150 minutes does no harm. These data are in general agreement with

those obtained by Sandstedt, although no loss of nitrogen on 150 minutes digestion with selenium was noted. There appears to be little difference in the minimum time requirement for correct results with selenium, selenium oxychloride, and mercuric oxide. With copper sulfate the time requirement is perhaps greater.

TABLE 1.

Effect of length of digestion period with A, gluten flour and single catalysts and B, ordinary flour and single catalysts

| | TIME DIGESTED | CATALYSTS AND AVERAGE PER CENT N_2 | | | |
		H_2O	Se	SeOCl$_2$	$CuSO_4 \cdot 5H_2O$
	minutes				
(A)	150	7.85	7.87	7.87	7.87
	120	7.87	7.84	7.84	7.87
	90	7.90	7.82	7.85	7.86
	75	7.89	7.88	7.85	7.84
	60	7.85	7.80	7.84	7.80
	50	7.86	7.80	7.81	—
	45	7.79	7.75	7.81	7.74
	30	7.74	7.69	7.76	7.67
(B)	150	1.60	1.62	1.63	1.63
	120	1.63	1.63	1.60	1.62
	60	—	1.61	1.60	1.62
	50	1.60	1.60	—	—
	45	1.60	—	1.60	1.62
	40	1.62	1.60	1.62	1.59
	35	1.58	1.60	1.59	—
	30	—	1.59	1.58	1.58

From the standpoints of speed and accuracy there appears to be little in favor of selenium or its oxychloride over the mercury catalyst and no significant difference between selenium and selenium oxychloride (Table 2, A and B).

The oxychloride is unstable on exposure to air. The acrid vapors which result during handling are disagreeable and no doubt poisonous. The cost per determination is four times that of an equivalent amount of the elemental selenium. On addition of either the element or its oxychloride to concentrated sulfuric acid a catalytic mechanism may be postulated in which selenious acid is in equilibrium with selenic acid. It appears, therefore, to be wholly unnecessary to employ a selenium compound in place of the stable, nonpoisonous element.

Table 2, A and B, summarizes the relative speeds of the several catalysts alone and in combinations with a gluten flour and an ordinary flour. All the results are the average of duplicate analyses. With the exception of one set of results of a 2-hour digestion with mercuric oxide

catalyst, the total time of digestion of the samples was approximately 50 per cent longer than the time required for clearing.

TABLE 2.

Relative speed of catalysts alone and in combinations with A, gluten flour and B, ordinary flour.

	CATALYSTS	CLEARING TIME	TIME DIGESTED	N_1
		minutes	*minutes*	*per cent*
(A)	None	100	100	7.70
	HgO	30	120	7.88
	HgO	30	45	7.79
	CuSO₄	40	60	7.79
	Se	25	38	7.75
	SeOCl₂	25	38	7.79
	HgO, +Se	20	30	7.85
	HgO, +SeOCl₂	20	30	7.84
	HgO, +CuSO₄	28	42	7.83
	CuSO₄, +Se	20	30	7.76
	CuSO₄, +SeOCl₂	20	30	7.84
	Se, +SeOCl₂	25	38	7.75
	HgO, +CuSO₄, +Se	20	30	7.88
(B)	None	95	100	1.60
	HgO	35	120	1.63
	HgO	35	50	1.60
	CuSO₄	31	46	1.62
	Se	25	40	1.60
	SeOCl₂	30	45	1.60
	HgO, +Se	25	37	1.61
	HgO, +SeOCl₂	25	37	1.61
	HgO, +CuSO₄	30	45	1.63
	CuSO₄, +Se	23	35	1.62
	CuSO₄, +SeOCl₂	20	30	1.62
	Se, +SeOCl₂	20	30	1.60
	HgO, +CuSO₄, +Se	20	30	1.62

It will be observed from the tables that all catalysts are far superior to the blank. The results from the K.-G.-A. digestions with mercuric oxide for 2 hours may serve as a basis for comparison with the other results.

With the ordinary flour, the time required for clearing was lessened when mixed catalysts were used and, in general, correct results were obtained when digestions were continued 10–15 minutes after clearing. With the gluten flour mixed catalytic combinations materially reduced the time required for clearing and a 30 minute digestion period was sufficient, or nearly so, when the following combinations were employed: mercuric oxide+selenium, mercuric oxide+selenium oxychloride, and mercuric oxide+selenium+copper sulfate hydrate.

SUMMARY

As a catalyst, selenium or its oxychloride has a slight advantage over copper sulfate but no advantage over mercuric oxide. Precipitated elemental selenium is more suitable and economical than selenium oxychloride.

A combination of selenium with mercuric oxide or with copper sulfate has a great advantage over any of these catalysts when they are used alone. Selenium with mercuric oxide appears to be the best combination.

A STATISTICAL TREATMENT FOR ESTABLISHING THE ACCURACY OF METHODS OF INTERPRETING ANALYTICAL RESULTS WITH SPECIAL REFERENCE TO CERTAIN EGG PRODUCTS

By F. A. VORHES, Jr. (U. S. Food and Drug Administration,* Washington, D. C.)

It is the purpose of this paper to present a systematic method of interpretation by comparison, applicable to a restricted type of sample which has been difficult to interpret by the usual methods. This type may be defined as a naturally occurring substance, separable into two parts, each part containing the same chemical elements in different proportions. One part of such a substance is usually less valuable than the other, and samples which are adulterated by an excess of the less valuable part are therefore likely to be encountered. Examples of such substances are eggs, separable into yolk and white; alfalfa, separable into leaf and stem; grains and spice seeds, separable into bran and cleaned seed; and pyrethrum, separable into flowers and stalk.

A procedure for obtaining a measure of the accuracy of comparison interpretations, based on accepted statistical methods of calculation is also presented.

As authentic samples are the basis of comparison, their reliability must be beyond question. They must be selected in such a way and consist of sufficient number as to be representative of all conditions which may be expected to vary their composition.

Mitchell[1] recently published a study of the composition of commercially fresh shell eggs. His data are probably the most complete, reliable, and extensive of their kind, and as such can be used advantageously as an example. Accordingly, the method will be developed as applying to eggs.

DEVELOPMENT

Given a mixture of two substances having a constituent in common, the fraction of this constituent in the mixture is shown by the equation—

$X = AY + BZ$, in which
$X =$ fraction of constituent in mixture;
$A =$ fraction of first substance in mixture;
$Y =$ fraction of constituent in first substance;
$B =$ fraction of second substance in mixture; and
$Z =$ fraction of constituent in second substance.

In applying this simple equation to a mixture of egg yolk and egg white let

$X =$ fraction of total nitrogen in the mixture;
$A =$ fraction of yolk in the mixture;
$Y =$ fraction of total nitrogen in the yolk;
$B =$ fraction of white in the mixture; and
$Z =$ fraction of total nitrogen in the white;

and let

$X' =$ fraction of water-soluble nitrogen in the mixture;
$Y' =$ fraction of water-soluble nitrogen in the yolk; and
$Z' =$ fraction of water-soluble nitrogen in the white.

Then $X = AY + BZ$, and $X' = AY' + BZ'$.

These are simultaneous equations of two unknowns. As such, they may be solved for each unknown as follows:[1]

Rearrange to $BZ = X - AY$, and $BZ' = X' - AY'$,

divide one by the other
$$\frac{Z}{Z'} = \frac{X - AY}{X' - AY'},$$

and simplify—

$$\frac{Z}{Z'} X' - \frac{Z}{Z'} AY' = X - AY, \quad \frac{Z}{Z'} AY' - AY = \frac{Z}{Z'} X' - X.$$

$$A = \frac{Z/Z'X' - X}{Z/Z'Y' - Y} = \frac{Z/Z'}{Z/Z'Y' - Y} X' - \frac{1}{Z/Z'Y' - Y} X.$$

If the fraction of yolk in the mixture is required in terms of **percentage**, multiply the equation by 100—

$$\% \text{ yolk} = 100 \left\{ \frac{Z/Z'}{Z/Z'Y' - Y} X' - \frac{1}{Z/Z'Y' - Y} X \right\}.$$

Substituting the average authentic data[2] for Z, Z', Y and Y'—

$$\% \text{ yolk}[3] = 100 \left\{ \frac{1.7221/1.6179}{1.7221/1.6179 \times .5079 - 2.6071} X' \right.$$
$$\left. - \frac{1}{1.7221/1.6179 \times .5079 - 2.6071} X \right\}$$

I. $= 48.39$ total nitrogen $- 51.51$ water-soluble nitrogen.

[1] Simultaneous equations have frequently been used in chemical calculations, for instance in gas analysis. Cf. p. 102 of Chemical Calculations by Salisburg & Lang (1918).
[2] The averages of the data presented by Mitchell, loc. cit.
[3] A formula similar in principle to this was given in an unpublished paper written in 1928 by Pappe and Smith on the estimation of fruit in pectin products.

By solving for B in an exactly analogous way, a formula for calculation of white may be obtained. The "A's" are cancelled and "B" is isolated.

$$B = \frac{Y/Y'}{Y/Y'Z' - Z} X' - \frac{1}{Y/Y'Z' - Z} X.$$

II.   % white $= 77.98\ W - 15.19\ N$.

The total egg is the sum of the white and the yolk, and therefore the sum of these two formulas gives a formula for total egg.

$$\% \text{ total egg} = 48.39\ X - 51.51\ X' + 77.98\ X' - 15.15\ X$$

III. $= 33.20\ N + 26.47\ W$.

Another formula of convenience is that for estimating the quantity of white in the total egg. The formula for percentage of white is divided by the formula for percentage of total egg and the quotient multiplied by 100.

IV.   % white in egg component $= \dfrac{77.98W - 15.19N}{33.20N + 26.47W} \times 100$.

ACCURACY OF FORMULAS

Since the constants of the formulas were obtained by the use of average authentic data, absolutely correct interpretations will be obtained only in the case of absolutely average samples. Average samples are not often encountered, and it has been the usual practice to use either the maximum or minimum values, from compilations of authentic data, which yield an interpretation more favorable to judging the compliance of the sample with stated requirements. Such practice very often gives too great tolerance because no recognition is taken of the correlation which often exists between constituents. If the error of the above formulas were calculated by the use of maximum or minimum data, the formulas would appear to be so inaccurate as to be useless. Such a conclusion is entirely erroneous, because the premises upon which it is based allow the assumption that maximum water-soluble nitrogen may occur simultaneously with minimum total nitrogen, and vice versa. Previous calculations made by the writer on Mitchell's data show that a high degree of correlation exists between water-soluble nitrogen and total nitrogen in eggs and that maximum values of one never occur simultaneously with minimum values of the other.

By a statistical treatment of the authentic data in connection with the formulas the error of estimation at any given probability may be calculated. As the probability approaches certainty, the error calculated approaches the maximum which may be expected under any conditions. Hence the error which may be considered maximum depends upon the probability which is selected as certainty.

For reasons which will appear later, the probability of 50 to 1 has been selected as certainty in this case. That is, it is assumed that the maximum error of the estimation is that error which will not be exceeded in more than one out of fifty estimations.

PROCEDURE FOR OBTAINING MAXIMUM ERROR

For each of the 42 samples of commercially fresh eggs analyzed by Mitchell, the result of formula I is calculated for 100 per cent white, and the following values are obtained:

$\sum R$ = sum of the results of the formula,

$\sum R^2$ = sum of the squared results of the formula.

Then the standard deviation of the results is calculated by the equation[1]—

$$S = \sqrt{\frac{\sum R^2}{N} - \left(\frac{\sum R}{N}\right)^2},$$

in which N = number of samples (42 in this case).

The standard deviation (S) gives that range about the mean within which 68.3 per cent of the results will lie. This value, multiplied by 2.33,[1] gives the range within which 98 per cent (49 out of 50) of the results will lie and is therefore the maximum error for estimating egg yolk in a sample which consists of 100 per cent white (0 per cent yolk).

The maximum error is then calculated for samples containing various percentages of white, the sum of percentage of white and percentage of yolk always being kept equal to 100. In this way the maximum error is expressed in terms of percentage of egg component, i.e., percentage of total egg present.

In the same manner the maximum errors of the other formulas are calculated. Table 1 gives the errors calculated.

TABLE 1

Maximum errors of formulas I to IV

WHITE IN EGG COMPONENT *per cent*	I	II	III	IV
		ERRORS (EXPRESSED AS PERCENTAGE OF EGG COMPONENT)		
100	±2.46	±7.36	±6.41	±2.42
80	2.44	5.84	5.14	2.40
50	3.06	3.83	3.49	2.61
20	3.95	2.84	3.15	2.73
0	4.93	3.38	3.82	3.17

The formulas are applicable to mixtures of eggs with other substances which do not contribute appreciably to the diagnostic determinations.

[1] A fundamental statistical formula and constants. See Methods of Correlation Analysis, by M. Ezekiel. John Wiley & Sons, New York (1930).

For instance, if salt were contained in a sample of liquid eggs, an estimation by the formulas would be perfectly practicable. However, if the maximum errors of the formulas had been expressed in terms of percentage of sample, the errors would vary with varying quantities of salt. Since the errors are in terms of percentage of egg component, and the egg component can be calculated within a known error, the errors of the other formulas can be readily converted into terms of percentage of sample.

It will be noted that formula IV is the most accurate. It is a type that probably can be used to great advantage in plant control laboratories where the percentage of total egg is known.

JUSTIFICATION OF THE PROBABILITY SELECTED AS CERTAINTY

The errors have been calculated on the basis that they would not be exceeded more than once in fifty times. However the error extends to its full range both to the positive and to the negative side of the true point. The chances, then, are equal that once in fifty times the error will exceed either the positive or negative range, but the probability that it will exceed one of the ranges only, is, of course, only half the probability that it will exceed either of the ranges. When the analyst, for example a regulatory analyst, is interested only in the error on one side of the true point, the probability is 100 to 1 that the allowance which he will make for interpretive error (by calculations similar to those above) is sufficient.

It should be remembered that the authentic data are based on samples of from 19 to 46 eggs, whereas samples of commercial egg products usually represent the mixture of several hundred eggs. Because such mixtures naturally reduce the variations due to inequalities in individual eggs, and samples from such mixtures will be closer to the average, it is likely that the accuracy of these formulas is greater than is indicated by pure statistics. Therefore, the error within which the calculations show the result to lie 99 per cent of the time is, to a very satisfactory degree of certainty, the true maximum error.

ADDITIONAL FORMULAS

It is possible that the first pair of diagnostic determinations chosen for calculation may not be perfectly satisfactory. The formulas derived from them may give too great errors. Other pairs of diagnostic determinations may often make a more accurate interpretation possible, and a third or fourth determination may conceivably be included to advantage. In addition, the sample to be analyzed must be considered. For instance, water-soluble protein of eggs is partially denatured in mayonnaise, and so far has resisted efforts to recover it in the quantities in which it was added in the preparation of known samples of mayonnaise. In any case, it is desirable that more than one formula be available for a check.

TABLE 2

Maximum errors of formulas V to XVI

WHITE IN EGG COMPONENT	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI
					ERRORS (EXPRESSED AS PERCENTAGE OF EGG COMPONENT)							
per cent												
100	±1.24	±7.30	±7.02	±1.26	±1.20	±7.28	±7.28	±1.21	±1.54	±7.23	±6.96	±1.53
80	1.41	6.17	5.61	2.03	1.37	5.71	5.46	1.80	—	—	—	—
50	2.30	5.19	4.03	3.48	2.23	3.71	3.41	2.53	2.05	3.49	3.13	2.52
20	3.29	5.87	4.58	5.16	3.20	2.49	3.27	2.34	—	—	—	—
0	4.08	7.16	5.65	7.17	3.98	2.65	4.05	2.64	3.54	2.86	3.44	2.86

The following formulas were calculated by using total P_2O_5 in connection with total nitrogen and water-soluble nitrogen.

V. % yolk = 75.69 P − 1.802 N.

VI. % white = 60.80 N − 114.59 P.

VII. % total egg = 59.00 N − 38.90 P.

VIII. % white in egg component $= \dfrac{60.80N - 114.59P}{59.00N - 38.90P} \times 100$.

IX. % yolk = 72.97 P − 1.849 W.

X. % white = 62.39 W − 22.91 P.

XI. % total egg = 60.54 W + 50.06 P.

XII. % white in egg component $= \dfrac{62.39W - 22.91P}{60.54W + 50.06P} \times 100$.

XIII. % yolk = 29.10 $(N+P)$ − 31.71 W.

XIV. % white = 71.76 W − 9.134 $(N+P)$.

XV. % total egg = 19.96 $(N+P)$ + 40.05 W.

XVI. % white in egg component $= \dfrac{71.76W - 9.134(N + P)}{19.96(N + P) + 40.05W} \times 100$.

These formulas and their maximum errors were calculated from Mitchell's data according to the procedure outlined. The errors are shown in Table 2.

AN ORTHODOX METHOD OF INTERPRETATION

Simple ratios are generally employed for comparison interpretations. For instance, the yolk in a sample of eggs may be estimated by the ratio of total P_2O_5 of the sample to the average total P_2O_5 of egg yolk. Such a procedure neglects the comparatively small quantity of P_2O_5 in egg white. The quantity of yolk having been estimated, the nitrogen of the sample due to yolk may be calculated approximately. Subtracting the latter figure from the total nitrogen of the sample, a figure representing the nitrogen due to white in the sample is obtained. Dividing this value by the average authentic nitrogen of white gives an estimate of the white present. Again by using Mitchell's data, mathematical expressions may be obtained as follows:

XVII.
$$\% \text{ yolk} = \frac{P_2O_5}{\text{Av. auth. } P_2O_5 \text{ of yolk}} \times 100 = \frac{P_2O_5}{1.3833} \times 100$$
$$= 72.29\ P.$$

$$\text{Nitrogen due to yolk} = \text{av. auth. N of yolk} \times \frac{P_2O_5}{\text{av. auth. } P_2O_5 \text{ of yolk}}$$

$$= \frac{2.6071}{1.3833}\ P_2O_5;$$

$$\text{nitrogen due to white} = N - \frac{2.6071}{1.3833}\ P_2O_5;$$

TABLE 3

Maximum errors of formulas XVII to XX

WHITE IN EGG COMPONENT	XVII			XVIII (ERRORS EXPRESSED AS PERCENTAGE OF EGG COMPONENT)			XIX			XX		
	AV. ERROR	RANGE OF ERROR ABOUT AV. ERROR	MAX. ERRORS	AV. ERROR	RANGE OF ERROR ABOUT AV. ERROR	MAX. ERRORS	AV. ERROR	RANGE OF ERROR ABOUT AV. ERROR	MAX. ERRORS	AV. ERROR	RANGE OF ERROR ABOUT AV. ERROR	MAX. ERRORS
per cent												
100	+2.96	±1.20	+4.16 to +1.76	−4.48	±7.98	+3.50 to −12.46	−1.52	±6.91	+5.39 to −8.43	−3.03	±1.27	−1.76 to −4.30
80	—	—	—	3.48	6.01	+2.53 to −9.49	1.18	5.54	+4.36 to −6.72	2.58	2.05	−0.53 to −4.63
50	1.45	2.54	+3.99 to −1.09	2.09	4.93	+2.84 to −7.02	0.65	3.85	+3.20 to −4.50	1.77	3.36	+1.59 to −5.13
20	—	—	—	0.84	5.55	+4.71 to −6.39	0.26	4.27	+4.01 to −4.53	0.82	4.78	+3.96 to −5.60
0	0.00	3.96	±3.96	0.00	6.85	±6.85	0.00	5.49	±5.49	0.00	6.84	±6.84

$$\% \text{ white} = \frac{N - \dfrac{2.6071}{1.3833}P_2O_5}{\text{Av. auth. N of white}} \times 100$$

$$= \frac{N - \dfrac{2.6071}{1.3833}P_2O_5}{1.7221} \times 100$$

XVIII. $\qquad = 58.07N - 109.44P.$

$\qquad\% \text{ total egg} = 58.07N - 109.44P + 72.29P$

XIX. $\qquad = 58.07N - 37.16P.$

XX. $\% \text{ white in egg component} = \dfrac{58.07N - 109.44P}{58.07N - 37.16P} \times 100.$

For the purpose of comparison the maximum errors of these formulas are given in Table 3.

Because the P_2O_5 of the egg white has been neglected in these formulas, an average error exists in all of them and hence the range of error is "canted" to one side or the other of the true point. It should be noted that in this case the errors must be considered in the opposite sense when used as corrections. For instance, the small quantity of P_2O_5 in white, when used in XVII, indicates that on the average there is 2.96 per cent yolk in pure white. Therefore 2.96 per cent yolk should be subtracted, not added, to such a calculation.

Total fat could have been used to a greater advantage than P_2O_5 in formulas XVII to XX, but it would not have permitted a true comparison with the previous formulas. In addition, the fat figure is worthless for interpretation of mayonnaise, a product upon which some of the formulas have been used.[1] It is possible that formulas combining water-soluble nitrogen and fat or a number of other combinations may be found more satisfactory for some samples than those presented. The purpose of these examples is merely to indicate a systematic procedure for use in deriving interpretive expressions and obtaining their accuracy.

USE OF MAXIMUM ERRORS

The most convenient way of expressing the error is usually by means of a chart. The charts given represent the data of Tables 1 to 3. Curves for formulas XIII to XVI are omitted for the sake of clarity and because their errors are not substantially less than those shown. The use of these charts is probably best explained by example.

Example

A contract for frozen eggs calls for a composition of not more than 64% white and not less than 36% yolk. The consignee analyzes a sample and obtains total P_2O_5, 0.429, and water-soluble nitrogen, 1.155 per cent. (These figures are based on a hypothetical mixture of eggs of composition of sample No. 12 in Mitchell's data,

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[1] Lepper and Vorhes, Mayonnaise Analysis and Interpretation Presented at Annual Meeting of Association of Official Agricultural Chemists in 1932. To be published later.

Symbol	Formula
O	I
◖	V
D	IX
+	XVII

Maximum Errors Expressed as Per cent of True Egg Component
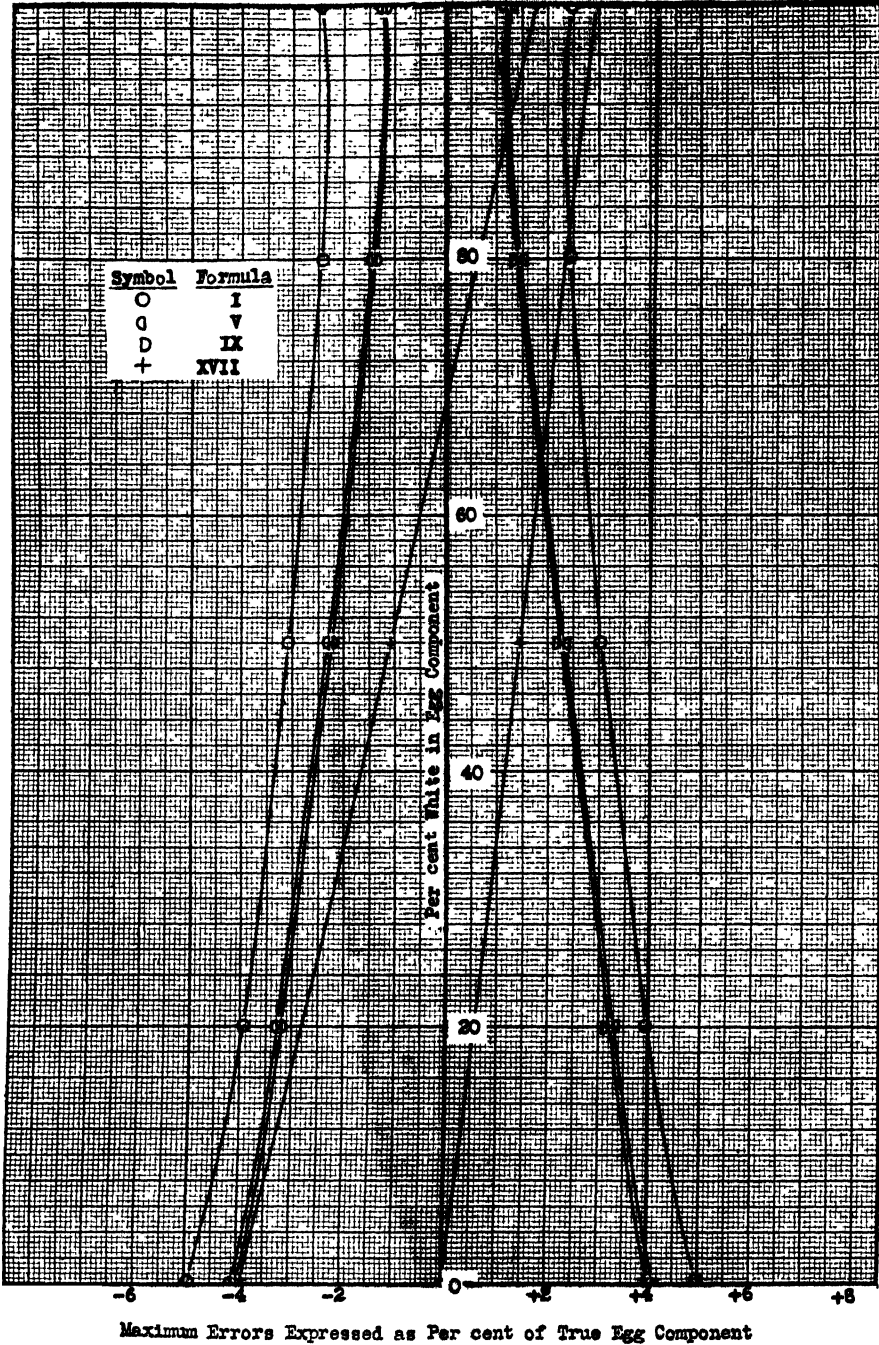
CHART I.—ERROR IN ESTIMATION OF EGG YOLK.
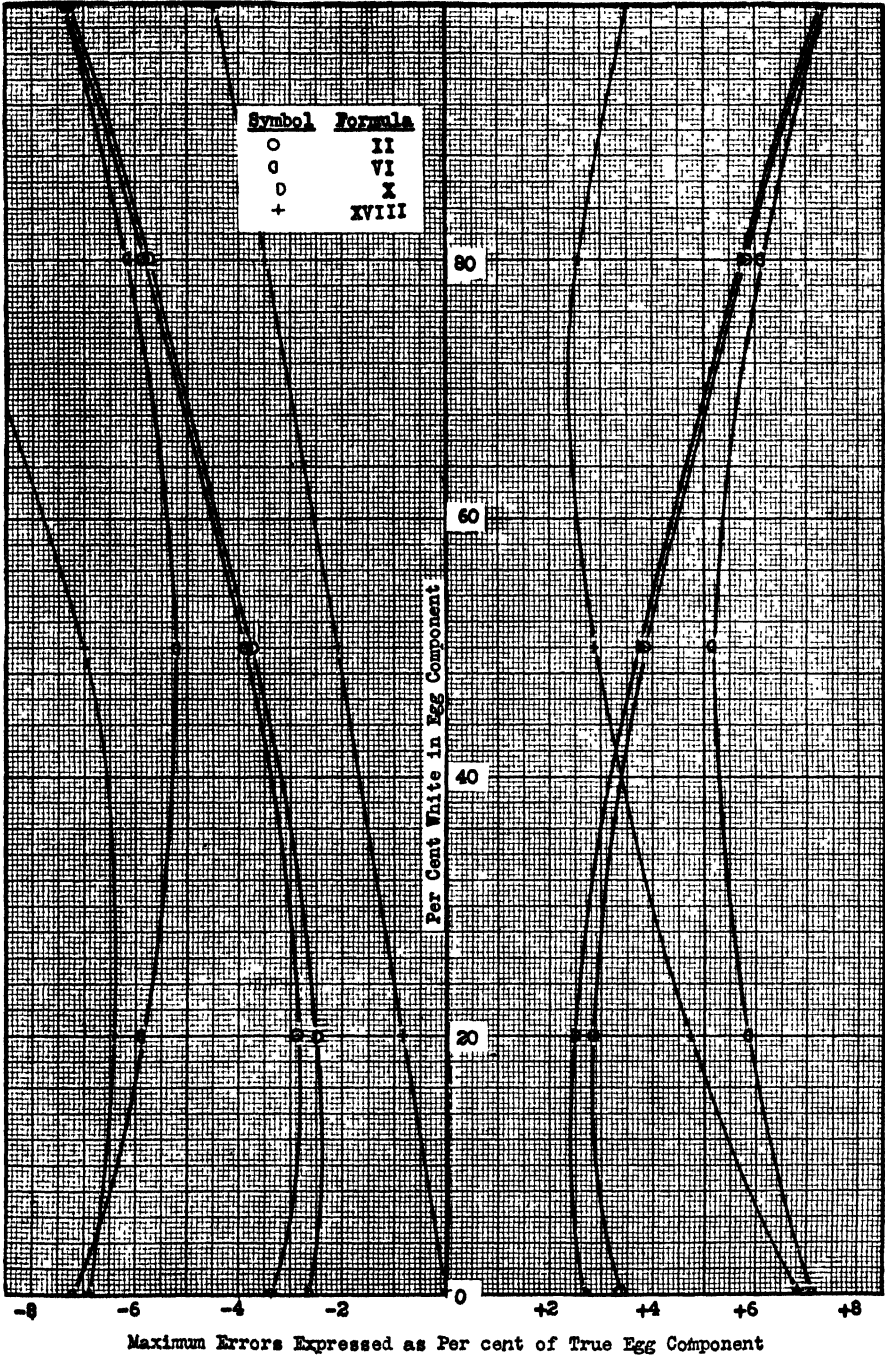
CHART 2.—ERROR IN ESTIMATION OF EGG WHITE.

Symbol	Formula
o	III
⊕	VII
⊅	XI
+	XIX
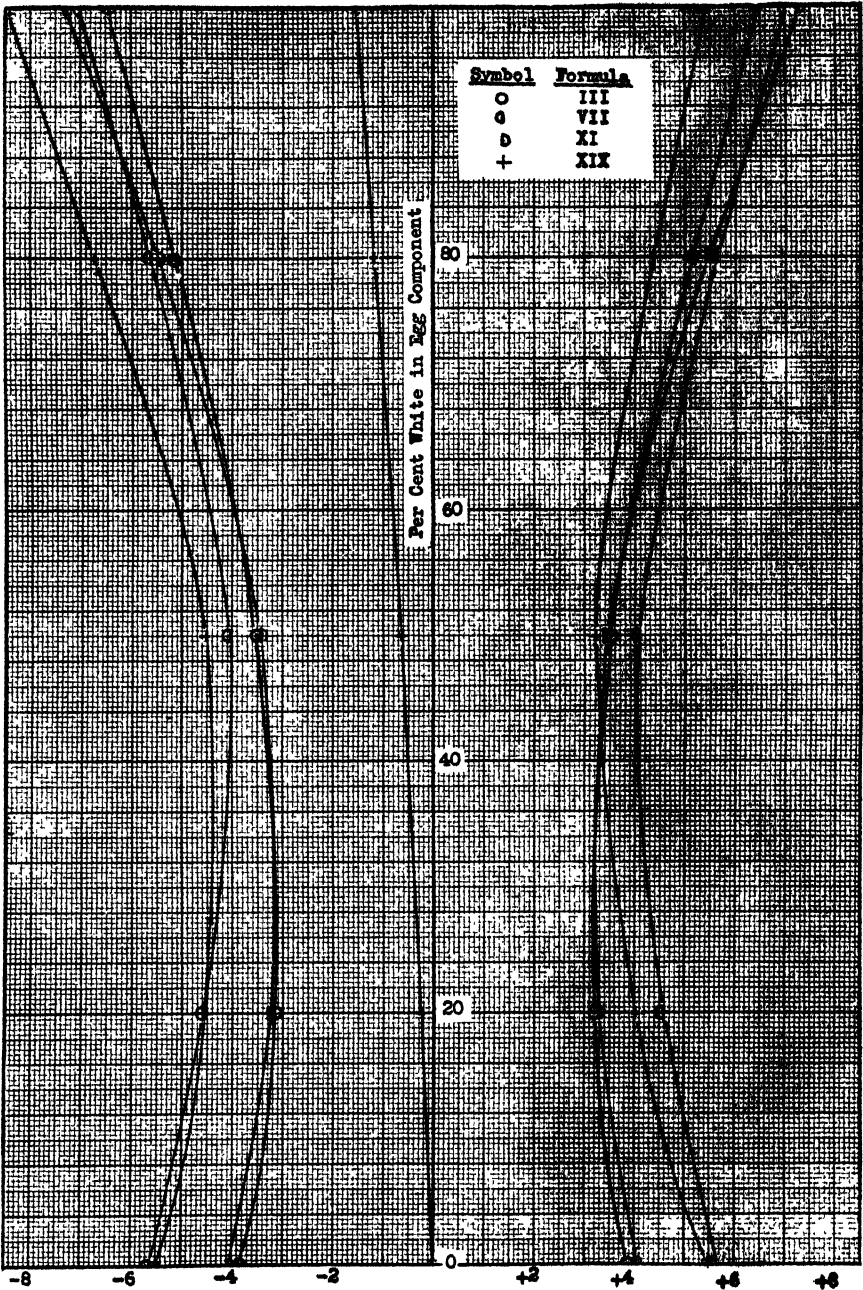
Maximum Errors Expressed as Per cent of True Egg Component

CHART 3.—ERROR IN ESTIMATION OF TOTAL EGG.

Symbol	Formula
o	IV
⊄	VIII
D	XII
+	XX
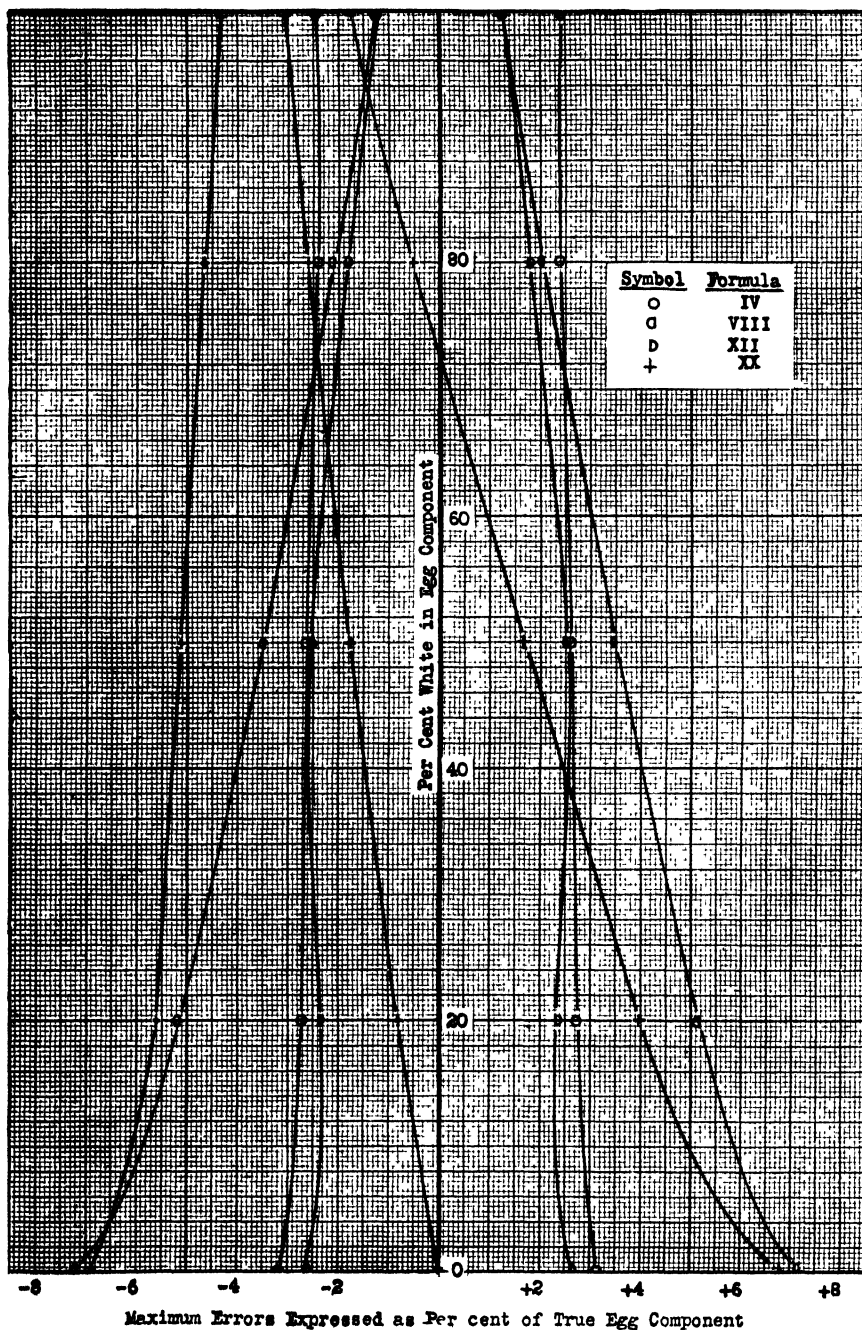
CHART 4.—ERROR IN ESTIMATION OF WHITE IN EGG COMPONENT.

which is assumed to be 60% white, 30% yolk and 10% water, salt or other "inert" material.)

Calculations are made as follows:

IX. % yolk = 72.97 × .429 − 1.849 × 1.155 = 29.17.

X. % white = 62.39 × 1.155 − 22.91 × .429 = 62.23.

XI. % total egg = 91.40.

XII. % white in egg component = 68.09.

From Chart 4 it is seen that the error for formula XII may be as much as ±2.1 per cent at 68.1 per cent white. Hence, the true percentage of white in the egg component lies somewhere between 66.0 and 70.2 per cent. Since the error of formula XI is seen from chart III to increase with an increasing percentage of white in egg component in the range 66.0 to 70.2 per cent, the safest procedure is to take the maximum value to obtain the error of XI. At this point the error of XI is ±4.7 per cent of the true egg component.

As the true egg component is not known, the calculated value is assumed to be in maximum error, and the range within which the true egg component (T. E. C.) will lie is found as follows:

$$\pm 91.40 \mp \text{T.E.C.} = .047 \times \text{T.E.C.}$$
$$\pm 91.40 = \pm \text{T.E.C.} + .047 \times \text{T.E.C.}$$
$$+91.40 = 1.047 \text{ T.E.C.}, \quad \text{T.E.C.} = 87.3\%$$
$$-91.40 = -.953 \text{ T.E.C.}, \quad \text{T.E.C.} = 95.9\%$$

The chances are greater than 100 to 1 that there is at least 100−95.9 or 4.1 per cent of something other than egg in the sample.

As the error of formula IX, as seen from Chart I, increases with decreasing white in egg component, the minimum value of white in egg component is taken in order to obtain the error of the calculation for yolk. The error is ±1.8 per cent of the true egg component at 66.0 per cent white in egg component. Since the highest value of total egg is 95.9 per cent, the largest error in estimation of yolk in terms of percentage of sample is 1.8 × 0.959 or 1.7 per cent. Therefore, the chances are less than 1 to 100 that the yolk is more than 29.2 + 1.7 or 30.9 per cent of the sample.

If the consignor is given credit for the maximum amount of total egg, the white must be at least 95.9−30.9, or 65.0 per cent of the sample.

The egg of contract specification is not more than 30.9/36 × 100 or 85.8 per cent of the sample, and the added white, if allowance for maximum total egg is given, is 95.9−85.8, or 10.1 per cent.

Table 4 gives a comparative presentation.

TABLE 4

	PRESENT	CALCULATED	ESTIMATE IF CREDIT FOR MAXIMUM ERRORS IS GIVEN CONSIGNOR
	per cent	per cent	per cent
Yolk	30	29.2	30.9
White	60	62.2	65.0
Total egg	90	91.4	95.9
White as percentage of egg component	66.7	68.1	—
"Inert"	10	8.6	4.1
Eggs of contract specification	83.4	81.1	85.8
Added white	6.6	10.3	10.1

The general rules for using such charts follow:

1. Obtain the maximum range for white in egg component in order that the errors of the other formulas may be located on their respective charts.

2. Obtain the maximum total egg in order that the errors may be converted to percentage of sample.

3. Obtain the maximum value of the component which may be estimated with the greatest accuracy.

(In the use of each of the foregoing rules, give the sample "the benefit of the doubt.")

4. Obtain the second component by difference. No "benefit of the doubt" can be given in this last case because it would conflict with benefits already given the other component and the total egg. It will be noted that the estimates of white and added white are not given as absolutely minimum or maximum values, but as minimum estimates only if the maximum values of yolk and total egg are admitted. If the maximum or minimum value of white is desired, a procedure analogous to that used for yolk may be employed.

On estimating the maximum or minimum values in this example, the errors of the various formulas have been to some extent interwoven or combined. For instance, maximum error for white in egg component is assumed when arriving at the error of the total egg and the yolk calculations. In addition, the maximum error of the total egg calculation is assumed in arriving at the error of the yolk calculation. Furthermore, these errors are so combined as to be non-compensating. Therefore, the probability that the results for yolk and total egg are maximum is extended still further beyond the 100 or 1 odds calculated from pure statistics.

SUMMARY

1. A method of interpretation by comparison is outlined; it is applicable to a type of sample on which determinations have been difficult to interpret.

2. Formulas obtained by this method and applying to some egg products are presented.

3. A method of estimating the maximum error of such formulas is suggested.

4. A measure of the accuracy of the formulas given is presented.

5. An orthodox method of interpretation and its accuracy as applied to some egg products is given.

6. Charts representing graphically the errors of the formulas given are presented, and the use of such charts is indicated.

ACKNOWLEDGMENT

DETERMINATION AND OCCURRENCE OF IODINE
IN PHOSPHATE ROCK

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In view of the biological importance of iodine an extensive investigation on the composition of natural phosphates, such as has been conducted in this Bureau[1] for several years, would hardly be complete without a survey of the iodine content of phosphate rock which, from the tonnage standpoint, is the most important of all primary fertilizer materials. The results of such a survey are presented in this paper.

ANALYTICAL METHODS

A. Furnace Method

Decomposition of the Sample.—The furnace method briefly outlined by McHargue[2] was used to decompose the sample and volatilize the iodine. The method used by the writers in making the complete determination is described briefly in the following paragraphs.

As measured by an electric pyrometer, the electric tube furnace, Hoskins type, gave a temperature of 900° C. after operating one hour and 1000° C. at the end of two hours. The quartz combustion tube was 18 inches long and its inside diameter was 1 inch. A smaller tube, 8 inches long, inside diameter about 0.5 inch, was sealed onto one end, making an angle of 90° with the larger tube. The large end of the combustion tube was fitted with a rubber stopper carrying a glass tube to admit oxygen. The tube was placed in the furnace so that the right-angle tube was about one inch from the end of the furnace. The oxygen used to sweep out the combustion gases was passed through a calcium chloride tower filled with glass wool, and two Milligan wash bottles charged with 40 per cent potassium hydroxide and concentrated sulfuric acid, respectively. The volatilized iodine was absorbed in 100 ml. of a 2.5 per cent solution of potassium carbonate contained in a Milligan wash bottle from which the head and top had been removed. The small end of the combustion tube dipped into the absorbent in such a manner that the gas stream passed into the spiral of the wash bottle.

A 5–10 gram sample (100-mesh) in a large porcelain boat was placed in the cold furnace, and after a slow stream of oxygen had been started through the tube the current was turned on. As a rule, the full current was employed from the beginning; however, with some materials the tempera-

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[1] *This Journal*, 11, 237 (1928); *Colloid Symposium Annual*, 7, 195 (1930); *Ind. Eng. Chem.*, 21, 1253 (1929); 22, 1392 (1930); 23, 1120 and 1413 (1931); 24, 86 and 1306 (1932).
[2] *This Journal*, 14, 138 (1931). Since this work was completed a paper by McHargue, Young and Roy, *Ind. Eng. Chem. Anal. Ed.*, 4, 214–6 (1932), has appeared, giving in some detail the method as applied to soils.

ture should be raised slowly in order to avoid the volatilization of large quantities of organic material. The total time of heating was usually about 3 hours, and the flow of oxygen was maintained until the furnace had cooled to 300–400° C. Finally, the boat was removed, and the inside of the quartz tube together with the contents of the absorption vessel was rinsed into a large evaporating dish.

Extraction of the Iodine from the Absorbent.—The contents of the dish were evaporated to a small volume on the water bath, then transferred to a small porcelain dish, evaporated to dryness in an air bath, and the residue was heated to 400° C. in a crucible furnace to char the traces of organic matter which are always present. The ignited residue was ground in a mortar and moistened with a quantity of water slightly less than that required to yield a pasty material (less than 1 ml.), and extracted four or five times by grinding with 5–10 ml. portions of 95 per cent redistilled alcohol and decanting through a 5 cm. filter paper. The filtered extract was evaporated to dryness in a 100 ml. beaker (tall form) without being allowed to boil.

Estimation of the Extracted Iodine.—The residue from the alcohol extract was taken up in about 5 ml. of water, and the solution was treated with 1 ml. of saturated solution of sulfur dioxide to reduce iodates, which otherwise would cause low results. Sulfuric acid (1+5) was now added in sufficient quantity to yield a final solution which, after being boiled, was barely acid to bromphenol blue paper. Four drops of the dilute acid was found to be ample for this purpose. The solution was then boiled 3 minutes to expel the excess sulfur dioxide, cooled to room temperature, and filtered. After the beaker and filter had been washed 2–3 times with small portions of cold water, the filtrate was brought to a volume of 10 ml. and transferred to a 12 cc. separatory funnel. The iodine was liberated with 1 ml. of a 2.5 per cent solution of sodium nitrite in the presence of exactly 1 ml. of pure carbon tetrachloride. The stoppered funnel was shaken 100 times and set aside for 1–2 minutes to allow the liquid layers to separate. The carbon tetrachloride layer was then drawn off into a 1 cc. glass-stoppered bottle and centrifugalized[1] (3000 R. P. M.) 3 to 5 minutes in order to obtain a clear solution. The clear solution was transferred to a microcolorimeter and compared with a standard[2] prepared in the same way from a known amount of standard potassium iodide solution. On account of the volatility of both iodine and carbon tetrachloride, it is necessary, especially in a warm room, to keep such solutions well covered until the readings can be taken.

Blank determinations on the reagents alone carried through all steps of the procedure showed only a very faint test for iodine.

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[1] McClendon, *J. Am. Chem. Soc.*, **50**, 1093 (1928).
[2] McHargue, *loc. cit.*

DISCUSSION OF THE FURNACE METHOD

When the procedure was used as described, 0.093 and 0.099 mg. of iodine were recovered from duplicate synthetic mixtures containing 0.1 mg. of iodine as potassium iodide and 5 grams of iodine-free tricalcium phosphate. That the small loss of iodine was due either directly or indirectly to the furnace treatment is to be inferred from the fact that 100 per cent recovery was obtained when the volatilization was omitted and the potassium iodide was added directly to the absorbent.

In all cases the iodine was completely volatilized from the sample. Satisfactory destruction of organic matter was effected in nearly all samples, a notable exception being Tennessee blue-rock phosphate No. 1049, which contains 1.5 per cent organic carbon. Although this sample contains less organic carbon than several of the other rocks, it is the only one which deposited soot in the tube during combustion. As noted by McHargue, traces of organic matter were observed in the absorbent after evaporation and ignition; however, with a few samples, particularly material relatively low in iron though not necessarily high in organic carbon, sufficient organic matter escaped combustion to give the absorbent a distinct color. As an example, Nauru Island phosphate No. 450 may be cited, in which case appreciable loss of iodine as a result of incomplete combustion undoubtedly occurred.

Unfortunately, this difficulty, as well as the one mentioned below, was not encountered until nearly all the samples had been analyzed. It was thought that the addition of a catalyst to the sample might correct this defect, and for comparison a few determinations were made, an intimate mixture of one gram of cupric oxide and 5 grams of rock being used. The results are given in Table 1. As indicated by the color of the material,

TABLE 1

Comparison of results obtained by acid-distillation and by the furnace method with and without copper oxide

				IODINE FOUND	
SAMPLE	SiO₂	Fe₂O₃	ACID-DISTILLATION METHOD	FURNACE METHOD	
				WITHOUT CuO	WITH CuO
	per cent	*per cent*	*p.p.m.*	*p.p.m.*	*p.p.m.*
450	—	< 0.30	—	16.5	27.5
985	0.39	—	124	122	—
932	5.01	0.69	138	130	153
589	—	1.26	—	93.2	107
906	7.11	3.42	17.7	28.2	22.0
910	7.28	2.59	4.3	10.9	6.6

much of the iron in Florida land pebble No. 910 and Tennessee brown rock No. 906 is present as the oxide; and in view of this, the limited data

indicate that it may be advantageous to use a catalyst with samples low in iron, especially oxides of iron. Considerable copper was volatilized during the combustion, and because of this, if a catalyst is to be used at all, ferric oxide would in all probability be more satisfactory. The use of a platinum spiral to produce a "hot spot"[1] in the quartz tube might also eliminate the difficulty of incomplete combustion and make the method applicable to all types of phosphate rock.

Iodine may also be lost from the absorbent during combustion, as well as during subsequent evaporation of the solution. Without entering into a discussion of the reactions which may be involved in this loss, it is sufficient to state that with a few samples, especially Nos. 932 and 450, the hardly mistakable odor of iodine was easily detectable when the absorbent was transferred to the evaporating dish. This loss persisted even when the absorbent was surrounded with an ice bath during combustion, and the odor was more pronounced when copper oxide was used with the sample. To insure complete reduction to iodide, McClendon,[2] in one instance, used a dilute solution of sodium sulfite between the combustion tube and the alkali absorbent. Although it was not tried by the writers, this addition to the absorption train would seem advisable.

B. *Acid-Distillation Method*

The sample was decomposed by distillation with sulfuric acid and hydrogen peroxide according to the procedure described by Glimm and Isenbruch.[3] With their absorption bulb, however, the maintenance of a steady flow of gas is very difficult, if not impossible, and for this reason the absorption vessel used in the furnace method was employed. The iodine was determined in the manner described in the furnace method.

On account of its high fluorine content, most phosphate rock, when heated in this way with concentrated sulfuric acid, yields large quantities of volatilized silica, much of which is deposited in the delivery tube of the apparatus. This necessitates frequent cleaning of the tube during distillation. The few results obtained by this method are given in Table 1. Although only about one-third of the iodine was recovered from potassium iodide, the results on the two samples of phosphate rock that are lowest in silica compare favorably with the values obtained by the furnace method. In the remaining cases, deposition of silica caused frequent interruptions during distillation, and low results were to be expected.

IODINE CONTENT OF PHOSPHATE ROCK

Forty samples of phosphate rock were analyzed for iodine, the furnace method as described being used. Of these samples, thirty were from de-

[1] Remington, McClendon and von Kolnitz, *J. Am Chem. Soc.*, **53**, 1245 (1931).
[2] McClendon and others, *J. Am. Chem. Soc.*, **52**, 541 (1930).
[3] *Biochem. Z.*, **207**, 368 (1929).

TABLE 2

New results on the iodine content of phosphate rock

SAMPLE	P₂O₅ per cent	IODINE p.p.m.	LOCATION OF DEPOSIT	SAMPLE	P₂O₅ per cent	IODINE p.p.m.	LOCATION OF DEPOSIT
			Florida Land-Pebble Phosphate				Tennessee White-Rock Phosphate
619	30.98	10.3	Nichols	1031	35.80	20.6	Godwin
910	31.09	10.9	Mulberry				Western Phosphates
947	31.28	13.1	Brewster	454	32.24	2.6	Conda, Idaho
439	33.22	15.4	Mulberry	973	32.53	1.9	Conda, Idaho
912	35.37	8.2	Mulberry	1009	31.39	0.8	Garrison, Montana
			Florida Hard-Rock Phosphate	1010	37.47	0.8	Garrison, Montana
771	31.25	72.0	Unknown	948	30.19	0.8	Cokeville, Wyoming
589	34.68	93.2	Floral City				Kentucky Brown-Rock Phosphate
434	35.33	87.0	Dunnellon	1235	21.19	9.5	Wallace
932	35.99	130	Dunnellon	1234	27.80	6.1	Wallace
			Florida Soft Phosphate				South Carolina Phosphate
728	31.80	88.3	Juliette	1139	26.92	3.7	Bulow Mines, Johns Island
			Florida Waste-Pond Phosphate	1138	27.85	13.9	Lamb's Mines, Charleston
726	23.48	50.8	Felicia				North African Phosphates
915	23.63	63.5	Dunnellon	552	27.55	4.7	Tunis: Gafsa
			Tennessee Brown-Rock Phosphate	562	28.59	3.7	Algeria: M'Zaita
56ᵃ	31.28	9.8	Unknown	561	28.66	8.0	Tunis: M'Dilla
762	33.73	18.1	Mountpleasant	1162	35.11	113	Morocco
1233	33.73	17.3	Mountpleasant				Island Phosphates
906	34.39	28.2	Wales	985	38.59	122	Curaçao Island
908	34.44	16.3	Mountpleasant	943	40.66	37.0	Curaçao Island
			Tennessee Blue-Rock Phosphate	450	38.92	16.5	Nauru Island
772	30.45	5.4	Glover	452	39.46	75.4	Christmas Island
930	30.97	6.4	Gordonsburg	451	40.32	18.3	Ocean Island
1049ᵇ	31.22	3.0	Boma	904ᶜ	54.51	<1.0	Connetable Islands

ᵃ Bureau of Standards standard sample No. 56.
ᵇ Variety of blue rock known as kidney phosphate.
ᶜ Essentially a phosphate of aluminum.

posits in the United States, four from deposits in North Africa, and six from island deposits. The results are given in Table 2, the iodine content being expressed in parts of iodine per million parts of rock. In order to indicate the grade of the rock, its phosphoric acid content, as P_2O_5, is also given. With samples containing less than 20–30 parts per million of iodine, duplicate results determined on different portions of the rock sample agreed well within 1 part per million. For larger quantities of iodine the difference between duplicate determinations was usually 3–5 parts per million. The deviation, however, was much greater in Florida hard rock and some of the island phosphates, reaching 20 parts per million in sample No. 932.

DISCUSSION OF RESULTS

Iodine was found in all samples analyzed (Table 2). The results vary within rather wide limits, ranging from 0.8 parts per million in Montana and Wyoming phosphates to 130 parts per million in one sample of Florida hard-rock phosphate. In the continental phosphates, however, the iodine content is fairly uniform in any given type of rock. That this is not true of the island phosphates is indicated by the results for the two samples of Curaçao rock, which contain 122 and 37 parts per million of iodine, respectively. Furthermore, the continental phosphates fall into two major groups,—one containing more than about 50 parts per million, the other containing less than about 30 parts per million of iodine. Florida hard-rock, soft and waste-pond phosphates, and Morocco rock comprise the first group, whereas the second includes all other samples from continental deposits. The iodine content of insular phosphates shows greater variation.

The results obtained in this investigation (Table 2) are, in general, higher than those previously reported[1] on the same type of rock. For example, the new values for Florida hard rock range from 72.0 to 130 parts per million, as compared with 54.5 and 92.5 parts per million found by Wilke-Dörfurt, Beck, and Plepp; and the average of two values for Tunis rock is 6.4 parts per million, while the average of the five previous figures is 5.9 parts per million. For a given type of rock the previous results, in most cases, either fall within the range set by the results in Table 2, or lie fairly close to the limits of the range. Thus, excluding sample 904, which is not a calcium phosphate, all the available figures may be brought together by taking the simple average of the results for each type of rock or group of deposits, as in the case of European phosphates. The summarized results are given in Table 3.

The results in Table 3 may be stated in a more compact form. Thus, the average iodine content of twelve samples of insular phosphates is

[1] Wilke-Dörfurt and others, *Z. anorg. allgem. Chem.*, 172, 344 (1928); Orr and Leitch, Med. Research Council Spec. Rept. Series No. 123 (1929); McHargue and others, *Am. Fert.*, 73, No. 10, 40 (1930).

36.1 parts per million. If the continental phosphates in two main groups are considered as before, the one containing more than 50 parts per million of iodine, the other containing less than about 30 parts per million, the average values of fourteen samples in the first group and forty-four in the second are, respectively, 90.5 and 9.4 parts per million of iodine. On the same basis, the average of the results on ten samples of Florida hard-rock, soft, and waste-pond phosphates is 78.2 parts per million, while the average of the twenty-six samples from other deposits in the United States is 9.2 parts per million of iodine. The average of the results on the seventy samples from all deposits is 31.3 parts per million.

TABLE 3

Summary of all results on iodine content of phosphate rock

SOURCE OR TYPE OF PHOSPHATE	NUMBER OF SAMPLES ANALYZED	IODINE	
		RANGE	AVERAGE
		p.p.m.	*p.p.m.*
Florida hard rock	7	54.5–130	89.2
Florida soft phosphate	1	—	88.3
Florida waste pond	2	50.8–63.5	57.2
Florida pebble	6	3.8–15.4	10.3
Tennessee brown rock	6	5.45–28.2	15.9
Tennessee blue rock	3	3.0– 6.4	4.9
Tennessee white rock	1	—	20.6
Kentucky brown rock	3	6.1– 9.5	7.4
Western United States	5	0.8– 2.6	1.4
South Carolina	2	3.7–13.9	8.8
Algeria	3	2.9–17.8	8.1
Egypt	2	1.2– 9.1	5.2
Morocco	2	53.5–113	83.3
Tunis	7	2.9–12.7	6.0
European deposits <about 30 p.p.m.	6	0.8–31.5	15.9
European deposits >50 p.p.m.	2	93.5–280	187
Curaçao Island	3	37.0–122	77.1
Nauru Island	5	1.1–19.1	11.5
Christmas Island	1	—	75.4
Ocean Island	1	—	18.3
Walpole Island guano	2	24.0–26.4	25.2

In conclusion a few comparisons may be indicated. Florida hard-rock phosphate, with an average of 89.2 parts per million of iodine, represents a fair average of all continental samples containing more than 50 parts per million. Similarly, Florida pebble, with an average iodine content of 10.3 parts per million, is only about one unit higher than the average of all continental samples containing less than about 30 parts per million of iodine, and it may thus be considered as typical of this group. It is of interest to note further that the average of 36.1 parts per

million for the island phosphates is near the average of 31.3 parts per million for all available results.

RELATION BETWEEN IODINE CONTENT AND GEOLOGIC AGE OF PHOSPHATE DEPOSITS

In Table 4 the available figures on the iodine content of phosphate rock are grouped, as far as possible, according to the geologic age of the deposits. Phosphate deposits belonging to the Tertiary period, especially

TABLE 4

Geologic age and iodine content of phosphate rock.

PERIOD OR EPOCH	SOURCE OR TYPE OF PHOSPHATE	NUMBER OF SAMPLES ANALYZED	IODINE	
			RANGE	AVERAGE
			p.p.m.	*p.p.m.*
Post Tertiary	Island Phosphates*	12	1.1–122	36.1
Tertiary		16	3.7–130	54.1
Pliocene	Florida land pebble*	5	8.2–15.4	11.6
Miocene	South Carolina*	2	3.7–13.9	8.8
Oligocene	Florida hard rock*	7	54.5–130	89.2
Eocene	Morocco[b]	2	53.5–113	83.3
Cretaceous	Egypt[c] and Tunis[d]	8	1.2–12.7	5.8
Carboniferous				
Permian	Western United States*	5	0.8– 2.6	1.4
Devonian	Tennessee blue rock*	3	3.0– 6.4	4.9
Ordovician	Tennessee and Kentucky brown rock*	9	5.6–28.2	13.1

References on geologic age of the phosphates.
* Mansfield, "Les Reserves Mondiales en Phosphates," Vol. I, p. 597, Bureau 14th Internat. Geol. Congress, Madrid, 1928.
[b] Despujols, *ibid.*, p. 597.
[c] Hume, *ibid.*, p. 565.
[d] Berthon, *ibid.*, p. 628.

the lower Tertiary, are richest in iodine, while the Quaternary island phosphates contain considerably less iodine and the Permian phosphates the least of all. Passing from Ordovician deposits through the Devonian to those of the Permian epoch, phosphate deposits become less rich in iodine. Somewhere between the latter epoch and the Cretaceous period, iodine enrichment began in phosphate deposits, the processes of enrichment becoming most active later in lower Tertiary time.

The overlapping ranges of the results indicate that the iodine content of phosphates depends also upon factors other than the age of the deposit. Thus, Fellenberg[1] found that, in general, fossiliferous deposits are richer in iodine than non-fossiliferous deposits—a result which was to be

[1] *Ergebnisse Physiol.*, 25, 176–363 (1926), p. 213.

expected in view of the fact that this element is concentrated in certain organs of both plants and animals. The average of Fellenberg's results on fossilliferous deposits is around 4 parts per million of iodine, whereas the average for non-fossiliferous deposits is only about one-half this amount. Although most phosphate deposits contain fossil remains in abundance, the bedded phosphates of Western United States, so far as observed,[1] are sparingly fossiliferous, and therefore may be compared in this respect with other phosphates, if the age factor be neglected. Thus, the average figure for iodine in these phosphates is 1.4 parts per million, which is only about one-fourth of that found for any other type of phosphate.

IODINE CONTENT OF PHOSPHATE DEPOSITS COMPARED WITH THAT OF OTHER DEPOSITS

Deposits of the Same Age.—Fellenberg's results,[2] among which no figures are given for phosphate rock, indicate that Jurassic deposits are richest in iodine, while those of the Cretaceous period are poorest. The

TABLE 5

Iodine content of rocks, minerals, soils, and water

| | NUMBER OF SAMPLES INCLUDED IN AVERAGE | IODINE | |
		RANGE	AVERAGE
		p.p.m.	*p.p.m.*
Igneous deposits			
Rocks	16	0.200– 0.810	0.358
Apatite	2	0.300– 0.440	0.370
Pyromorphite	2	0.400–10.000	5.200
Sedimentary deposits, including	12	0.120– 8.850	0.930
Quartzites	3	1.100– 8.850	4.550
Anhydrite	1	—	0.300
Gypsum	2	0.250– 0.270	0.260
Calcite	1	—	0.230
Limestone	1	—	0.440
Marble	3	0.120– 0.600	0.390
Dolomite	1	—	0.320
Phosphate rock	71	0.8 –280	31.3[a]
Soils			
New Zealand	473	0.0 –70.0	2.7[b]
Kentucky: Mercer County	10	3.135– 8.250	5.252[c]
Ocean and Sea Water	13	0.0434–0.695	0.0542[d]

[a] This paper (collected data).

[b] Hercus, Benson and Carter, *J. Hyg.*, **24**, 321–402 (1925), p. 347.

[c] McHargue, Young and Roy, *Ind. Eng. Chem. Anal. Ed.*, **4**, 214 (1932).

[d] Reith, *Rec. trav. chim.*, **49**, 142 (1930).

Other results by Fellenberg, *Ergebnisse Physiol.*, **25**, 176–363 (1926), p. 209.

[1] Mansfield, U. S. Geol. Survey Professional Paper, **152**, 361 (1927).
[2] *Loc. cit.*, p. 210.

variation, however, is not so great as in phosphate rock deposits (Table 4). Considering averages, for example, his results give 1.120 parts per million of iodine for four post-tertiary samples, 0.935 for ten samples from the Tertiary, 0.380 for four from the Cretaceous, 2.370 for eight from the Jurassic, and 0.550 parts per million for six samples from the Triassic. Among the individual results the highest is 9.2 parts per million, while the next highest is only 2.3 parts per million. These figures, on comparison with the results in Table 4, show that, in general, phosphate rock is much richer in iodine than other deposits of the same geologic age.

Deposits in General.—Fellenberg's results on the distribution of iodine in rocks, as well as the results of a few other writers and those obtained in this investigation, are summarized in Table 5. These results indicate that iodine has accumulated to a much greater extent in phosphate rock than in other rock deposits.

SUMMARY

The dry-distillation and acid-distillation methods for the determination of iodine in phosphate rock are discussed. Dry distillation is shown to be the better procedure.

Iodine was determined on forty samples of natural phosphates from various deposits in the United States and elsewhere. The results range from 0.8 to 130 parts per million. The results, with the data found in the literature, are summarized (1) according to the type of phosphate, and (2) according to the geologic age of the deposits.

The iodine content of phosphate rock is considerably higher than that of other rock deposits.

ESTIMATION OF POTASSIUM BY TITRATION OF THE COBAL-TINITRITE WITH POTASSIUM PERMANGANATE

By P. L. HIBBARD and P. R. STOUT (Division of Plant Nutrition, University of California, Berkeley, California)

Some of the advantages of the method described are rapidity, simplicity, accuracy, non-interference of small amounts of difficultly soluble substances such as calcium sulfate or silica, and suitability for making many determinations at one time. It is applicable to quantities of 0.1–5.0 mg. of potassium as found in almost any kind of material, and is particularly suitable for estimating potassium in soil extracts, plant materials and biological solutions. The method, however, is not suitable for estimating quantities of potassium over 5 mg. or for a few infrequent determinations of smaller amounts as it requires some experience and technical skill, also special equipment for filtering and washing the precipitate and for accurately and rapidly measuring the standard solutions used.

The method has given very satisfactory results in the writers' and also in neighboring laboratories during more than two years. It is similar to the procedure described by Kramer and Tisdall,[1] but it can be more easily adapted to varying quantities of potassium by a modification in the titration procedure. A skilled operator can filter and titrate 10 to 15 determinations in one hour.

The method follows:

REAGENTS

(1) *Cobaltinitrite solution.*—

    A. 113 grams of cobaltous acetate dissolved in 300 cc. of hot water and 100 cc. of glacial acetic acid.

    B. 200 cc. of sodium nitrite dissolved in 400 cc. of hot water.

Mix equal parts of cooled A and B solutions, force air through the mixture until red fumes cease to be evolved, and protect from light and ammonia fumes. If the mixture is not quite clear, filter before using.

(2) *Potassium permanganate, 0.05 N.*—Must be free of oxides of manganese.

(3) *Sodium oxalate, 0.05 N.*—Dissolve in water and add 6 cc. of sulfuric acid (1+1) per liter. The 0.05 N solutions keep fairly well for some weeks, but the 0.01 N should be made fresh every day by dilution from the 0.05 N solution. Both standard solutions are dispensed by means of autozero, 10 cc. burets graduated in 0.05 cc. Before beginning each titration, fill both burets to zero.

(4) *Sulfuric acid (1+1).*—Free from oxidizable matter by heating with permanganate.

(5) *Acetic acid.*—2% by volume.

(6) *Talc powder for filtering.*—Elutriate to remove the very fine and very coarse particles. Then remove any oxidizable matter by the action of permanganate plus sulfuric acid. For use, add water to make about 5 grams of talc in 100 cc. of the suspension; 5 cc. of this is about the right amount for one filter on the Jena sintered glass filter funnel 39 G3, porosity 3. (When the water has been drawn through the filter by suction, the talc should form a firm coherent layer on the sintered glass from 0.4 to 0.7 mm. thick. It should not break up when liquid is poured upon it, and it must retain all the yellow precipitate on its surface. None of the precipitate should be allowed to go through and collect on the surface of the sintered glass. With properly prepared talc and a clean filter it should be possible to filter and wash the precipitate from a single sample in one minute. If much more time is required, something is wrong.)

PROCEDURE

Take a quantity of material containing 0.2–2.0 mg. of potassium. (Less than 0.2 mg. cannot be estimated with accuracy and over 2.0 mg. tends to make the titration tedious and perhaps less accurate. With 0.5 mg. of potassium, the accuracy in titration ordinarily is plus or minus 1 to 2 per cent.) To remove any organic matter and ammonium salts that may be present, add to the solution 1 cc. of sulfuric acid (1+1), evaporate to dryness, heat gently on a hot plate till white fumes begin to appear, add from a pipet a few drops of concentrated nitric acid, heat till dry, and ignite to whiteness. Extract the residue with dilute hydrochloric acid and filter, if necessary, into a 50 cc. Pyrex beaker.

If the composition of the solution to be analyzed is unknown, it is advisable to make a rough test in order to decide what aliquot to use. To 2 cc. of the solution,

[1] *J. Biol. Chem.*, 46, 339 (1921).

add 2 cc. of cobaltinitrite reagent. If a precipitate forms immediately, an aliquot of 2–10 cc. may be sufficient; if no precipitate forms within 5 minutes, take an aliquot of 25 cc. or more.

Add a little hydrochloric acid, evaporate to dryness, and redissolve the residue in 5 cc. of the 2 per cent acetic acid. (If the residue contains less than 0.2 mg. of potassium, it is better to use only 2 cc. of the 2 per cent acetic acid to dissolve it because the precipitate, potassium cobaltinitrite, is slightly soluble.)

To the prepared solution add 5 cc. of the cobaltinitrite reagent. (It is very important to have the ratio of volume of solution to volume of reagent 1:1 in all cases in order to obtain constant composition of the precipitate.) Cover the beaker and let stand at room temperature for 12–15 hours. A longer period will do no harm, but a shorter period may have a tendency toward erratic and lower results.

For filtration use preferably Jena glass filter funnels holding about 25 cc., with sintered glass filter disks No. 39 G3, porosity 3 and 20 mm. diameter of filter plate. For rapid work use a vacuum equal to 18–20 inches of mercury. When the filter is in position on the suction bottle, *not connected* to suction, pour into the filter 5 cc. of the prepared talc suspended in water. After 10–20 seconds turn on the suction, and when the water has been sucked out, wash the filter with a small quantity of the 2 per cent acetic acid. (The talc should form a smooth layer, 0.2–0.5 mm. thick, on the glass filter disk. This film of talc is the real filtering surface, and its careful preparation is an essential factor. The liquid should pass through the filter at the rate of 40–50 cc. per minute.)

Pour into the filter the liquid and precipitate of the potassium cobaltinitrite. Suck through all the liquid, then wash out the beaker and filter with the 2 per cent acetic acid. Wash till entirely free of the cobaltinitrite reagent.

When the amount of potassium in the aliquot taken is very small, 0.05 mg. for example, which will require in the titration only about 1 cc. of 0.01 N potassium permanganate, fairly good results may be had by mixing a little especially purified talc with the precipitate before pouring it onto the talc filtering surface. However extreme care must be taken to exclude any oxidizable substance other than the precipitate itself, which might reduce the permanganate.

Wash the precipitate with the 2 per cent acetic acid until the yellow color of the reagent disappears, using a few cc. at a time; then rinse both beaker and filter at least four times with the same solution. Finally, wash once with pure water to remove any acetic acid that might remain and reduce the permanganate. The total washings should not exceed 30–40 cc.

Rinse the precipitate with the talc into the original beaker, using 5–15 cc. of water. Any yellow precipitate remaining on the glass filter disk shows that there was not enough talc on the filter or that the precipitate was too fine and not properly formed. In this case, the result will be low. Clean the filter funnel after each filtration by inverting it on a one-holed rubber stopper connected with the suction and running water in through the stem.

Into a 200 cc. Pyrex Erlenmeyer flask, run a few cc. of the standard potassium permanganate and add 2 cc. of the sulfuric acid (1+1) and 10–15 cc. of water. Use 0.01 or 0.05 N potassium permanganate, according to the amount of precipitate to be handled. Set on a small hot plate, heated by an ordinary Bunsen burner, and when the liquid boils pour into it some of the suspension of talc and yellow precipitate from the beaker. If the potassium permanganate appears to be nearly used up, add more before putting in more of the yellow precipitate. (It is essential to have an excess of potassium permanganate, otherwise the result will be low.) Finally pour the boiling mixture back into the beaker to oxidize the adhering precipitate and heat the beaker a moment to complete the oxidation. Run into the pink liquid some of the standard sodium oxalate till colorless, then titrate back with the potassium

permanganate to faint pink, which should be permanent for several minutes. (Less than 1 cc. of the oxalate should be sufficient to reduce the excess of potassium permanganate added.)

One cc. of the 0.01 *N* potassium permanganate is equivalent to 0.062 mg. of potassium. The exact factor is found by making a determination on a known amount of potassium, exactly as described above. This should be done frequently in order to check the standard solutions and method of operation.

A suitable amount of potassium for standardizing is 0.5 mg. As this quantity usually requires 8.0 or 8.1 cc. of 0.01 *N* potassium permanganate, the factor will be 0.0625 or 0.0618.

Collect the watery liquid with the talc in a large flask and allow to settle. When the water is poured off the talc is ready for use again.

The table below presents a comparison of results obtained by the method described and by the chloroplatinate method. The solution used represents 0.05 gram of plant material per cc.

SAMPLE NO.	VOLUMETRIC METHOD			GRAVIMETRIC METHOD		
	ALIQUOT USED	0.05 *N*[*] KMnO₄ USED	K in PLANT MATERIAL	ALIQUOT USED	K₂PtCl FOUND	K IN PLANT MATERIAL
	cc.	cc.	per cent	cc.	gram	per cent
a	2.00	12.60	3.84	20.0	0.2409	3.87
b	2.00	12.80	3.91	20.0	0.2380	3.83
c	2.00	6.15	1.88	20.0	0.1190	1.91
d	2.00	7.95	2.43	20.0	0.1507	2.43
e	2.00	14.10	4.30	20.0	0.2656	4.28
f	2.00	6.30	1.93	20.0	0.1183	1.91

[*] Known amount, 4 mg. of K =13.10; (4.0/13 1) =0.305 mg. of K per cc. of 0.05*N* MnO₄.

THE PRECIPITATION OF MILK PROTEINS BY MEANS OF TRICHLOROACETIC ACID[1]

By George P. Sanders (Bureau of Dairy Industry, U. S. Department of Agriculture, Washington, D. C.)

The increasing use of the trichloroacetic acid method for removing the proteins of milk, as a means of determining the quantities and chemical relationships of the various protein and non-protein fractions and of the minerals, led to a study of the efficiency of this acid as a precipitant of milk proteins. The varying results and incomplete precipitation which have recently been reported by Moir[2] and Rauschning[3] have probably resulted from the fact, pointed out long ago by Simon,[4] that a high concentration of this acid in the milk-acid mixture is necessary. It was therefore decided to use varying concentrations and amounts of acid and to

compare this procedure with the commonly used method of Folin and Wu,[1] which was devised for studies of blood. The Folin and Wu procedure was later simplified by Haden.[2]

Greenwald[3] precipitated the proteins of blood by means of a picric-acetic acid mixture and determined the "acid-soluble" phosphorus, sulfates, calcium, sodium, and potassium in aliquots of the filtrate. Denis and Minot[4] precipitated the proteins of milk by means of copper sulfate and heat, and Simon[4] used various reagents, including tannic, trichloroacetic, phosphotungstic, and metaphosphoric acids. Trichloroacetic acid was used by Greenwald[5] for the precipitation of blood proteins in order to determine the amounts of non-protein nitrogenous constituents present; by Roe and Kahn[6] for preparing protein-free filtrates for blood calcium determinations; by Moir[7] for studying the amounts of certain protein fractions in milk; by the writer[8] for preparing filtrates for determinations of calcium, magnesium, and acid-soluble phosphorus in milk; and later by Rauschning[9] for milk calcium determinations. Greenwald states that the lipids of blood, together with all the protein material, are precipitated by trichloroacetic acid, and that this acid does not split off nitrogen from the proteins of blood.

The mechanism of milk casein precipitation by trichloroacetic acid has been explained by Loeb and Loeb,[10] Loeb,[11] and Wilson.[12] They demonstrated that the solubility of casein in certain acids is directly proportional to the swelling of the casein molecule, and that this characteristic is dependent upon the Donnan membrane equilibrium. A decreasing order of solubility was shown in solutions of the following acids in the order named: phosphoric, hydrochloric, nitric, sulfuric, trichloroacetic. These writers stated that the force of cohesion in casein particles in the presence of the trichloroacetate anion is so great that it can not be overcome by the osmotic pressure which results from the Donnan equilibrium, and hence swelling and solution in this acid are greatly inhibited.

The isoelectric precipitation of casein has been described by Michaelis and Pechstein,[13] who showed that the point of minimum solubility of this protein occurs at a hydrogen-ion activity of 2.5×10^{-5} (pH 4.6).

Palmer and Scott[14] and Sebelien[15] discussed the precipitation of albumin,

[1] *J. Biol. Chem.*, **38**, 81 (1919).
[2] *Ibid.*, **56**, 469 (1923).
[3] *Ibid.*, **38**, 439 (1919).
[4] *Ibid.*, 453.
[5] *Ibid.*, **21**, 61 (1915).
[6] *J. Biol. Chem.*, **81**, 1 (1929).
[7] *Loc. cit.*
[8] *J. Biol. Chem.*, **90**, 747 (1931).
[9] *Loc. cit.*
[10] *J. Gen. Physiol.*, **4**, 187 (1921).
[11] Proteins and the Theory of Colloidal Behavior, 2nd ed., p 354. McGraw-Hill Book Co., New York (1924).
[12] Physical Chemistry of Casein. Casein and Its Industrial Applications (Sutermeister, E.), p. 49; Chemical Catalog Co., New York (1927).
[13] *Biochem. Z.*, **47**, 260 (1912).
[14] *J. Biol. Chem.*, **37**, 271 (1919).
[15] *Z. physiol. Chem.*, **13**, 135 (1889).

and Palmer and Scott described the effect of milk preservatives upon albumin precipitation. The work of Van Slyke and Bosworth[1] indicated that 100 per cent of the nitrogen other than that contained in the casein and albumin occurs in the serum filtrate when milk becomes "sour" due to the development of lactic acid.

EXPERIMENTAL

In the quantitative determination of the two proteins which occur in largest amounts in milk, namely casein and albumin, the methods outlined by the Association of Official Agricultural Chemists[2] (Casein, Method 1; Albumin, Method 1) were used. The factor 6.38 was used for converting nitrogen to protein.

In studying the efficiency of trichloroacetic acid as a precipitant of

TABLE 1

Precipitation of milk proteins by trichloroacetic acid. *

SAMPLE NO.	GRAMS PROTEIN PRECIPITATED PER 100 CC. OF MILK							
	PROTEIN PRECIPITATED BY TRICHLOROACETIC ACID				PROTEIN PRECIPITATED BY HEATING THE NEUTRALIZED TRICHLOROACETIC ACID FILTRATES			
	CONCENTRATION OF ACID, BY WEIGHT				CONCENTRATION OF ACID, BY WEIGHT			
	0.06 N (1.0%)	0.10 N (1.6%)	0.60 N (10%)	2.43 N (40%)	0.06 N (1.0%)	0.10 N (1.6%)	0 60 N (10%)	2.43 N (40%)
1	2.77	2.86	3.20	3.24	0.37	0.31	0.03	0.01
	2.75	2.88	3.18	3.22	0.36	0.29	0.03	0.00
2	2.80	2.85	3.25	3.25	0.45	0.40	0.01	0.00
	2.79	2.85	3.23	3.25	0.42	0.38	0.03	0.00
3	2.93	3.00	3.30	3.42	0.36	0.31	0.10	0.00
	2.94	3.02	3.27	3.44	0.37	0.31	0.09	0.00

* 1 part acid to 1 part milk.

TABLE 2

pH values of mixtures of equal quantities of cows' milk and trichloroacetic acid solutions of different concentrations.

SAMPLE	ORIGINAL MILK	NORMALITY OF ACID SOLUTION ADDED						
		0.05	0.06	0.10	0.20	0.40	0.609	2.43
Mixed*	6.54	4.84	4.45	2.90	1.45	0.96	0.83	0.31
Holstein	6.61	4.78	4.31	2.79	1.42	0.93	0.78	0.30
Jersey	6.60	4.87	4.54	3.09	1.50	0.99	0.84	0.31

* Averages of several samples during the summer months.

[1] New York Agr. Expt. Sta. Tech. Bull. 48 (1916).
[2] *Methods of Analysis*, A.O.A.C., 215 (1930).

nitrogenous compounds in milk, equal parts of milk and acid solution were used in the early experiments. The normalities of the acid solutions were varied as shown in Tables 1 and 2, and the amounts of nitrogen precipitated in each case were determined by the Kjeldahl method. Blank tests were run on all reagents. The heat-coagulable protein (albumin) in the filtrates, shown in the last four columns in Table 1, was determined by neutralizing the filtrates with dilute ammonia-free sodium hydroxide and carrying out albumin analyses by the heat-coagulation method mentioned previously.

In later work four volumes of 10 per cent (approximately 0.60 N) trichloroacetic acid solution were mixed with one volume of milk. No heat-coagulable protein was found in the filtrates from these mixtures, and the filtrates gave negative results when subjected to the biuret test.

The trichloroacetic acid method was compared with the tungstic acid method of Folin and Wu.[1]

The data in Table 1 indicate that as the concentration of the acid solution is increased the quantity of albumin precipitated increases and that precipitation of albumin is practically complete when 2.43 N (approximately 40 per cent) acid solution is used.

The casein is precipitated at its isoelectric point (pH approximately 4.6), and it apparently remains undissolved as the acidity is further increased. The quantities of a 0.20 N trichloroacetic acid solution required to increase the hydrogen-ion concentrations of 100 cc. of each of the milks to pH 4.6 were as follows: Jersey, 31.2 cc.; mixed, 28.2 cc.; Holstein, 26.5 cc.

The analyses show that 0.60 N (approximately 10 per cent) or 2.43 N (approximately 40 per cent) trichloroacetic acid solution, when mixed with milk in the proportion of 1:1 by volume, precipitates slightly more protein than is represented by the sum of the casein and albumin. The work of Palmer and Scott,[2] Simon,[3] Sebelien,[4] and others[5] indicates that the heat coagulation method may not precipitate all the albumin in milk.

The data in Table 3 indicate that trichloroacetic acid is almost as efficient as is tungstic acid as a precipitant of nitrogenous compounds in milk. The quantities of nitrogen in the filtrates in the case of either acid correspond in general with figures for the non-protein nitrogen content of cows' milk, which Denis and Minot[6] found to be 18.0 to 38.0 mg. per 100 cc. Van Slyke and Bosworth[7] found 49.0 mg. of nitrogen, other than that contained in casein and albumin, in 100 cc. of milk.

Four parts of 10 per cent trichloroacetic acid solution with one part of milk was somewhat more effective for precipitating nitrogenous com-

[1] *Loc. cit.*
[2] *Loc. cit.*
[3] *Loc. cit.*
[4] *Loc. cit.*
[5] Walker and Cadenhead, *J. Ind. Eng. Chem.*, 6, 573 (1914); Gibson, *This Journal*, 9, 233 (1926).
[6] *Loc. cit.*
[7] *Loc. cit.*

TABLE 3

Comparison of the efficiency of tungstic and trichloroacetic acids as precipitants of milk proteins.

MILK SAMPLE NO.	GRAMS NITROGEN PER 100 CC. OF MILK						
				TOTAL NITROGEN MINUS CASEIN AND ALBUMIN NITROGEN	NITROGEN IN FILTRATE USING		
	TOTAL NITROGEN	CASEIN NITROGEN	ALBUMIN NITROGEN		TUNGSTIC ACID	TRICHLOROACETIC ACID	
						1 PART 10% ACID TO 1 PART MILK	4 PARTS 10% ACID TO 1 PART MILK
1	0.4502	0.3367	0.0685	0.0450	0.0250	0.0349	0.0277
	0.4490	0.3366	0.0680	0.0444	0.0256	0.0340	0.0270
2	0.5676	0.4350	0.0780	0.0546	0.0277	0.0481	0.0306
	0.5670	0.4330	0.0784	0.0556	0.0273	0.0490	0.0310
3	0.5866	0.4545	0.0768	0.0553	0.0298	0.0540	0.0324
	0.5880	0.4553	0.0783	0.544	0.0300	0.0560	0.0328
4	0.5743	0.4452	0.0760	0.0531	0.0309	0.0536	0.0325
	0.5758	0.4480	0.0754	0.0524	0.0315	0.0530	0.0330
5	0.5415	0.4270	0.0658	0.0487	0.0285		0.0302
	0.5415	0.4277	0.0674	0.0464	0.0280		0.0300
6	0.5740	0.4496	0.0752	0.0492	0.0283		0.0302
	0.5720	0.4500	0.0750	0.0470	0.0277		0.0299
7	0.6095*	0.4812*	0.0793*	0.0490*			

* Calculated from figures of Van Slyke and Bosworth (*loc. cit.*).

pounds than was one part of 10 per cent or one part of 40 per cent acid solution with one part of milk, particularly when milk containing a high percentage of total solids was analyzed. The efficiency of the procedure is influenced by the concentration of trichloroacetic acid.

The pH values of the milk-acid mixtures were as follows: one part milk to 4 parts 10 per cent trichloroacetic acid, pH 0.56 to 0.53; one part milk to one part 40 per cent acid, pH approximately 0.30; tungstic acid procedure of Folin and Wu, pH 2.57 to 2.51.

The completeness of precipitation was found in every instance to be dependent upon the buffer value of the milk. The higher this value, the greater is the concentration of acid required to produce maximum precipitation. The buffer values of representative samples of Jersey, Holstein, and mixed milks, for two different acids, are shown in Fig. 1.

In order to furnish a comparative evaluation of the buffer capacities of the three milks (Fig. 1) for the two acids, the quantities of a 0.2004

N solution of each acid required to change the hydrogen-ion concentration of 100 cc. of each milk from its original pH to pH 3.50 were calculated. These data follow:

	HCl	CCl$_3$COOH
	cc.	*cc.*
Jersey Milk	52.6	54.2
Mixed Milk	48.9	50.4
Holstein Milk	46.5	48.0

It should be mentioned that hydrochloric acid precipitates the casein in the region of the isoelectric point of casein (pH approximately 4.6), and that additional hydrochloric acid causes no further protein precipitation, but rather effects the resolution of casein. Trichloroacetic acid precipitates the casein at the isoelectric point, and additional trichloroacetic acid precipitates albumin and other milk proteins.
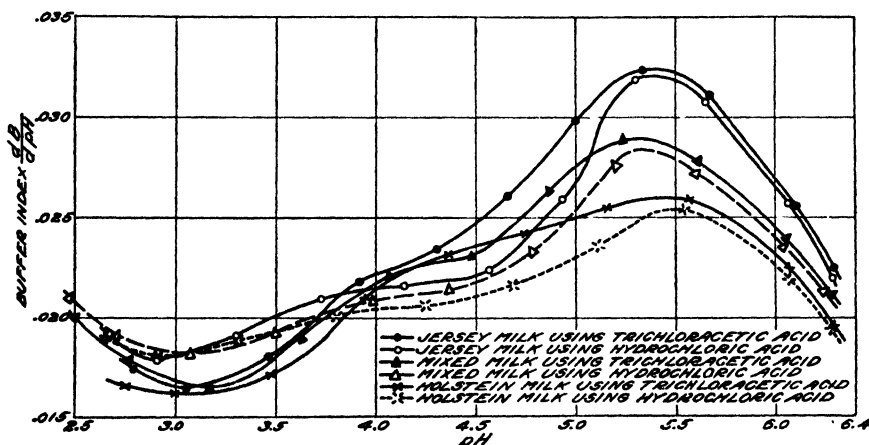
FIG. 1.—BUFFER INTENSITIES OF JERSEY, MIXED, AND HOLSTEIN MILKS, IN VARIOUS PH REGIONS, WITH HYDROCHLORIC AND TRICHLOROACETIC ACIDS.

Because the data given in Table 3 were not sufficiently complete to allow calculation of the percentage distribution of the protein among the various precipitated fractions, another experiment was carried out wherein the total nitrogen content of the sample, as well as that of each separated fraction and of each filtrate, was determined. The results are given in Table 4.

Tungstic acid was slightly more effective than trichloroacetic acid as a precipitant. It seems probable, therefore, that tungstic acid, which precipitates a greater quantity of nitrogenous compounds than does trichloroacetic acid, in addition to precipitating the protein materials, may also precipitate some substance of a basic, non-protein nature. There re-

mains also the possibility that trichloroacetic acid precipitates all the proteins except a very small fraction of undetermined nature.

TABLE 4

Relative amounts of nitrogenous compounds precipitated, by various methods, from 100 cc. of milk.

	NITROGEN	PROTEIN N.×6.38	PERCENTAGE OF TOTAL
	grams	*grams*	
Total nitrogen and protein in 100 cc. of milk......................	0.516	3.29	100.0
Casein (by acetic acid coagulation)	0.387	2.47	75.0
Albumin (by heat coagulation)....	0.068	0.43	13.2
In filtrate from casein and albumin	0.062		12.0
Precipitated by 40% trichloroacetic acid(1:1)*.....................	0.478	3.05	92.6
Heat coagulable protein in filtrate from 40% trichloroacetic acid precipitation (1:1)*...............	0		0
Nitrogen in filtrates:			
Tungstic acid (Folin and Wu method)................ . .	0.0278		5.39
10% trichloroacetic acid (1:1)*...	0.0325		6.30
40% " " (1:1)*...	0.0324		6.28
10% " " (4:1)† .	0.0322		6.24

* 1 part acid to 1 part milk.
† 4 parts acid to 1 part milk.

SUMMARY

Trichloroacetic acid, when used in the ratio of 4 parts of 10 per cent acid to 1 part of milk, was found to be almost as effective as tungstic acid for precipitating nitrogen compounds in milk.

The trichloroacetic acid method has the following advantages: (1) only one reagent instead of two is added to the milk; (2) the casein particles are much coarser and filtration is much more rapid; (3) a larger quantity of filtrate is secured from a given quantity of mixture.

The completeness of precipitation by trichloroacetic acid is dependent upon the concentration of the acid in the milk-acid mixture. In the experiments reported a mixture of 4 parts of 10 per cent trichloroacetic acid solution with 1 part of milk produced the most complete precipitation.

The possibility of using this method for determining the non-protein nitrogen constituents of milk, as was done by Greenwald in the case of blood, is suggested. Among these constituents, as reported in the literature, are ammonia, amino nitrogen, urea, preformed creatinine, creatine, and uric acid.

NOTES

Recovery of Platinum in Potash Determinations[1]

Every laboratory using platinum as a reagent plans to recover this salt from the residues, and many suggestions for handling these residues may be found in the literature. Practically all these procedures reduce the platinum to the metallic form, which separates as a black insoluble residue. This residue is filtered out, purified, washed, and finally dissolved in aqua regia to reform the platinum chloride.

Blair[2] reduces the platinum by allowing the alcoholic residues to stand in the sunlight until all the platinum has separated out. McDowell[3] and McDermott[4] prefer to use aluminum. Hough[5] uses granular magnesium. Treadwell and Hall[6] suggest the use of sodium formate. The writers have used C.P. granular zinc with satisfactory results.

The following directions, formulated from suggestions by McDowell and Hough, are presented. This process is applicable when it is desired to recover the alcohol from the platinum washings, as it allows its recovery in a condition to be used again for platinum determinations. If platinum is present during distillation some of the alcohol is oxidized to acetone and to acetaldehyde.

Treat alcoholic washings and residues at once with a strong solution of ammonium chloride in water to precipitate the platinum as the yellow ammonium chloroplatinate. If the ammonium chloride washings have been run into the residues the platinum is already precipitated, in which case test the clear alcoholic solution by adding an extra cc. of strong ammonium chloride solution. Pour alcoholic liquid, together with the platinum precipitate, upon a filter and allow to drain. Set the filtrate aside for the recovery of the alcohol.

Dissolve the ammonium chloroplatinate from the filter by means of hot water. Also dissolve the potassium chloroplatinate that was weighed up in the potash determination from the crucibles with hot water and combine with the solution of the ammonium chloroplatinate. Add 50 cc. of strong hydrochloric acid for each liter of the solution, and drop pieces of mossy or granular, chemically pure zinc into the solution. (In place of zinc, granular magnesium or strips of pure aluminum may be used.) If the liquid is not colorless when the metal has dissolved, add more metal until all the platinum is precipitated. Then collect the precipitated metallic platinum on an ashless filter and wash thoroughly to remove impurities. Burn the filter with the adhering platinum in a crucible and treat the residue, consisting of metallic platinum, the residue of the filter, and a small quantity of the adhering metal used in the precipitation, with successive portions of hydrochloric acid (1+5) to dissolve the adhering metallic impurities. Wash, dry, and weigh the platinum residue. Dissolve the platinum in nitrohydrochloric acid, evaporating off the excess of acid. Add strong hydrochloric acid and again drive off the excess of acid. Repeat this three times, when all nitric acid should be removed. Pass chlorine gas in excess

[1] By L. D. Haigh and A. R. Hall, University of Missouri, Columbia, Missouri.
[2] *Ind. Eng. Chem.*, 2, 102 (1910).
[3] *Ibid.*, 10, 128 (1918).
[4] *J. Am. Chem. Soc.*, 32, 336 (1910).
[5] *Ind. Eng. Chem., Anal. Ed.*, 1, 162 (1929).
[6] Analytical Chemistry, 6th ed., Vol. 1, p. 285.

through the solution, evaporate to a low bulk, and make up the solution to such a volume that 1 cc. will contain 0.100 gram of platinum. Filter, and the solution is ready for use.

When the alcohol is recovered a small quantity of black precipitate is sometimes found in suspension at the end of the distillation owing to the dissolved platinum present in the alcoholic filtrate. Pour the liquid residue in the flask through a filter and add the residue to the other residues of precipitated platinum.

Extractor for Large Quantities of Organic Material[1]

In a chemical investigation requiring the extraction of large quantities of organic material with a volatile solvent, no extractor of sufficient size was available. The apparatus shown was assembled from material available in the laboratory.

The illustration is in a large measure, self explanatory. The body of the extractor is an inverted bell jar, closed with a desiccator top. The charge to be extracted (A), is supported by a porcelain desiccator plate (C), on which rests a layer of absorbent cotton (B).
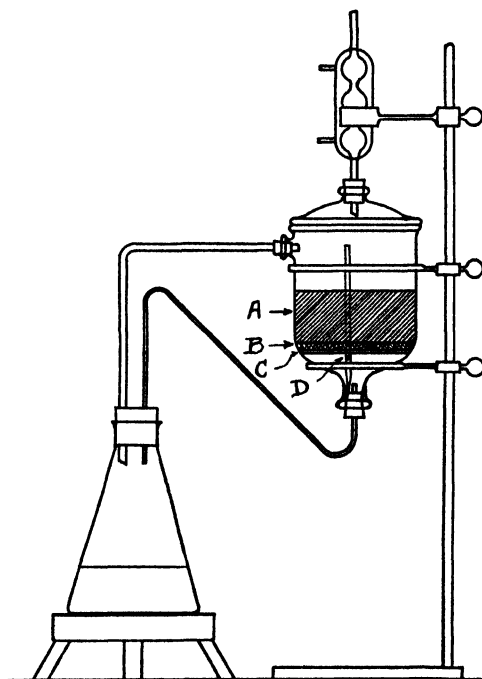
An important item in the assembly is the vent tube (D), without which the siphon will not flow intermittently, but will drip continuously. The conditions are somewhat different from those in an ordinary Soxhlet ex-

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tractor in which the material is held in a paper capsule. In the case of the Soxhlet there is an air vent between the capsule and the side of the extractor, while in the extractor described the material is in contact with the glass and cuts off the free flow of air, necessitating the use of the vent tube.

Loss of solvent through the ground joint between the bell jar and desiccator top can be prevented by moistening the ground surfaces with a viscous liquid that is immiscible with the solvent. By using petroleum ether (boiling range 35°–40° C.) and sealing with a few drops of glycerine very little loss occurs during a 24-hour extraction.

The capacity of this extractor is limited only by the size of the bell jar and flask used in its assembly. The one employed by the writer satisfactorily handled charges ranging up to 750 grams.

FIRST DAY

MONDAY—MORNING SESSION

REPORT ON INSECTICIDES AND FUNGICIDES

By J. J. T. GRAHAM (Insecticide Control, Food and Drug Administration, Washington, D. C.), *Referee*

The referee continued the study of methods for the analysis of Bordeaux-lead arsenate mixtures. Collaborative study of Method II[1] was omitted this year at the request of its sponsor, in order to give him time to study modifications for its improvement.

An electrolytic method proposed by C. G. Donovan[2] was studied in comparison with the official method.

Two samples of Bordeaux-lead arsenate were selected for collaborative study. Sample 1 was prepared by the referee from high grade materials. The content of lead oxide and copper, calculated from an analysis of the Bordeaux mixture and lead arsenate used in its preparation, was 22.35 and 12.53 per cent, respectively. Sample 2 was a commercial product and was considered to be a representative type of material. A careful analysis by the referee, using the official method, gave 17.60 and 14.45 per cent, respectively, for lead oxide and copper.

These samples were sent to the collaborators with directions to make determinations of lead and copper in Bordeaux-lead arsenate mixture by the method described on p. 39 of *Methods of Analysis*, A.O.A.C., 1930, or on pp. 51 and 52 of the 1925 edition (this method was designated Method I) and by the Donovan method (designated Method II).

The collaborative results are given in Table 1.

The results reported by the collaborators are very good. They show that there is little choice between the methods so far as accuracy is concerned, although the results by Method II are slightly more uniform. Method II requires less manipulation and less time to complete than Method I, and would undoubtedly be the choice of analysts in laboratories supplied with the proper electrical equipment.

It is recommended[3] that Method II be adopted as official, first action, and that it be further studied.

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[1] *This Journal*, 15, 172 (1932).
[2] *Ibid.*, 289.
[3] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 42 (1933).

TABLE 1

Collaborative results—Bordeaux mixture with lead arsenate

ANALYST	LEAD OXIDE				COPPER			
	SAMPLE 1		SAMPLE 2		SAMPLE 1		SAMPLE 2	
	METHOD I	METHOD II	METHOD I	METHOD II	METHOD I	METHOD II	METHOD I	METHOD II
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
A. Alter	22.34	22.36	17.63	17.76	12.49	12.45	14.40	14.42
New York	22.19	22.44	17.71	17.73	12.44	12.41	—	14.41
Average	22.27	22.40	17.67	17.75	12.47	12.43	14.40	14.42
J. C. Bubb	—	22.51	—	17.77	—	12.44	—	14.41
New York	—	22.65	—	17.68	—	12.49	—	14.42
	—	22.47	—	—	—	12.43	—	—
Average	—	22.54	—	17.73	—	12.45	—	14.42
C. F. Cressy	—	22.49	17.62	17.61	—	12.48	—	14.44
New York	—	—	17.63	—	—	12.48	—	14.35
Average	—	22.49	17.63	17.61	—	12.48	—	14.40
C. G. Donovan	22.29	22.36	17.63	17.74	12.54	12.45	14.28	14.47
Washington, D. C.	22.40	22.33	17.47	17.75	12.60	12.53	—	14.48
Average	22.35	22.35	17.55	17.75	12.57	12.49	14.28	14.48
J. J. T. Graham	22.33	22.37	17.58	17.53	12.50	12.48	14.36	14.50
	22.27	22.46	17.61	17.63	12.60	12.48	14.54	14.50
Average	22.30	22.42	17.60	17.58	12.55	12.48	14.45	14.50
E. C. Haas	22.46	22.17	17.81	—	—	12.52	—	14.41
New York	22.45	22.48	17.73	—	—	12.50	—	14.44
	22.43	—	17.75	—	—	12.47	—	—
Average	22.45	22.33	17.76	—	—	12.50	—	14.43
J. P. Henry	—	22.38	—	17.75	—	12.50	—	14.32
Washington, D.C.	—	22.47	—	17.70	—	12.55	—	14.45
	—	—	—	17.70	—	—	—	14.50
Average	—	22.43	—	17.72	—	12.53	—	14.42
W. L. Miller	22.27	22.34	17.52	17.71	12.42	12.24	14.37	14.41
New York	22.23	22.35	17.58	—	12.45	12.37	14.42	—
Average	22.25	22.35	17.55	17.71	12.44	12.31	14.40	14.41
General average	22.33	22.41	17.64	17.70	12.51	12.46	14.40	14.43
Theoretical	22.35	22.35	—	—	12.53	12.53	—	—
Average deviation from the mean	0.08	0.08	0.07	0.05	0.06	0.05	0.06	0.04

REPORT ON FLUORINE COMPOUNDS

By G. A. SHUEY (University of Tennessee, Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee*

An attempt is being made to adapt present methods to the determination of small quantities of fluorine compounds on the surface of foliage and fruits. Some attention has also been given to methods for the removal of barium fluosilicate and aluminum sodium fluoride (cryolite) spray residues from fruits prior to analysis.

The method developed by H. H. Willard and O. B. Winter[1] was studied. This method is based on the principle that fluoride solutions can be titrated with standard thorium nitrate solution when zirconium-alizarine is used as indicator. Any interfering elements may be eliminated from the solution to be titrated by volatilizing the fluorine as hydrofluosilicic acid.

DIRECT TITRATION OF PURE FLUORIDE SOLUTIONS

In all the analyses presented, final titrations were carried out under conditions identical with those used in standardizing the thorium nitrate. The factors of volume and amounts of indicator are of especial importance.

In order to study the accuracy of the method, 1, 2 and 3 cc. portions of 0.1 N sodium fluoride solution were diluted to 50 cc. Aliquots of 10 cc. were then titrated with standard thorium nitrate solution, three drops of zirconium-alizarine being used as indicator. The concordant results obtained for the three concentrations are given in Table 1.

TABLE 1
Titration of pure fluoride solutions

0.1 N NaF USED	STANDARD Th(NO$_3$)$_4$ required*	FLUORINE	
		PRESENT	FOUND
cc.	*cc.*	*mg.*	*mg.*
1	7.0	1.9	1.883
2	14.0	3.8	3.766
3	21.5	5.7	5.783

* 1 cc. standard Th(NO$_3$)$_4$ equivalent to 0.269 mg. fluorine.

VOLATILIZATION OF FLUORIDE AS HYDROFLUOSILICIC ACID

Interfering elements absent.—Quantities of 1, 2 and 3 cc. of 0.1 N sodium fluoride were distilled in the presence of perchloric acid, glass beads, and a few quartz crystals. The distillate, which amounted to about 75 cc., was rendered alkaline to litmus, evaporated, and made up to 50 cc. Aliquots of 10 cc. were rendered acid with hydrogen chloride and titrated with standard thorium nitrate, three drops of zirconium-alizarine indicator being used. The results are given in Table 2.

[1] Manuscript by courtesy of O. B. Winter. Paper now published in *Ind. Eng. Chem Anal. Ed.*, 5, 7 (1933), and in *This Journal*, 16, 105 (1933).

TABLE 2

Titration of fluoride solutions recovered by distillation

0.1 N NaF USED	STANDARD Th(NO₃)₄ REQUIRED	FLUORINE	
		PRESENT	FOUND
cc.	cc.	mg.	mg.
1	7.0	1.9	1.883
2	13.75	3.8	3.698
3	21.75	5.7	5.850

Interfering elements present.—Ten grams of finely ground peach leaves was treated with 0, 1, 2 and 3 cc., respectively, of 0.1 N sodium fluoride and an excess of saturated limewater. After being mixed, the material was dried and carefully ignited. The ash was transferred to a 50 cc. Pyrex distilling flask and distilled with perchloric acid. The distillate was evaporated and titrated as in the former case. The results are given in Table 3.

TABLE 3

Titration of fluoride solutions recovered from plant ash by distillation

0.1 N NaF USED	STANDARD Th(NO₃)₄ REQUIRED	FLUORINE	
		PRESENT	FOUND
cc.	cc.	mg.	mg.
0	0.00	0.00	0.00
1	6.25	1.9	1.681
2	13.25	3.8	3.564
3	19.25	5.7	5.178

DETERMINATION OF FLUORINE SPRAY RESIDUES ON FOLIAGE

Samples of peach leaves were taken just before each subsequent application of barium fluosilicate spray. Ten-gram samples were analyzed for fluorine exactly as described for the synthetic samples. Results are shown in Table 4.

TABLE 4

Titration of fluoride solutions obtained from spray residues

DATE SAMPLED	STANDARD Th(NO₃)₄ REQUIRED	FLUORINE FOUND
	cc.	mg.
6–7–32	3.0	0.807
7–11–32	6.8	1.829
7–6–32	9.5	2.555
8–10–32	31.5	8.473

As indicated by the data in Table 1, the method is accurate for the direct titration of pure fluoride solutions. Fluorine was quantitatively recovered by distillation from pure fluoride solutions as shown in Table 2. The data of Table 3, however, indicate that only about 90 per cent of the added fluorine was recovered from plant ash. The incomplete recovery

should not be interpreted to mean that the method does not register all the fluorine present in the plant ash, as some of the fluorine may have been lost during ignition. This factor remains to be studied. The data of Table 4 show a progressive accumulation of fluorine on foliage with each successive application.

The method is rapid and appears to lend itself to the analysis of fluorine compounds in general. Further study with respect to the recovery of fluorine from plant ash, etc, should be made.

<div align="center">RECOMMENDATIONS[1]</div>

It is recommended—

(1) That the Willard and Winter method for the determination of fluorine be studied further.

(2) That collaborative and experimental study of that method and also of the present tentative method for the determination of fluorine be conducted.

<div align="center">REPORT ON CAUSTIC POISONS</div>

<div align="center">By J. J. T. GRAHAM (Insecticide Control, Food and Drug Administration, Washington, D. C.), *Referee*</div>

The products covered by the Federal caustic poison act are common acids and acid salts, common bases, hypochlorites, silver nitrate, and salts of oxalic acid.

A method for the determination of carbolic acid in the presence of other phenols has been adopted by the Association. For most of the remaining products there are well-known standard methods. Therefore the need for collaborative study was not sufficiently urgent to request it at a time when most laboratories were operating with reduced forces.

It is recommended that the position of Referee on Caustic Poisons be discontinued and that the subject of caustic poisons be given to the Referee on Insecticides and Fungicides.

<div align="center">REPORT ON SUGARS AND SUGAR PRODUCTS</div>

<div align="center">By R. T. BALCH (Bureau of Chemistry and Soils, Department of Agriculture, Washington, D. C.), *Referee*</div>

The progress of the work on the analysis of sugars and sugar products will be discussed in detail by the associate referees. The recommendations for further study offered by these associate referees are concurred in by the referee.

Of interest to the Association should be the report of the recent meeting of the International Commission for Unifying Methods of Sugar Analysis. Doubtless many of the conclusions and methods adopted by this impor-

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[1] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 42 (1933).

tant Commission will have a direct bearing upon the methods of this Association.

It has been called to the attention of the referee that there is some ambiguity regarding the proper selection and use of clarifying agents. In order to clear this situation, the Referee offers some recommendations. These recommendations have been published.[1]

REPORT ON HONEY

By H. A. SCHUETTE[2] (Department of Chemistry, University of Wisconsin, Madison, Wisc.), *Associate Referee*

The recommendation was made in last year's report[3] that methods for determining the diastatic activity of honey be studied. The standardization of a method for this determination seemed desirable not only on theoretical grounds but also for very practical reasons in view of the stand taken by German food control agencies that a genuine honey, if not overheated, must meet a specified minimum before importation is permitted.

The three methods available when this study was begun[4] were those of Gothe,[5] Fiehe and Kordatski,[6] and Lothrop and Paine.[7] Lothrop and Paine pointed out that it was necessary to work under pH-controlled conditions (5.26–5.30) in order to obtain a high degree of accuracy in this determination. The substance of their procedure may be synoptically summarized as follows: (*a*) preparation of a series of twelve phosphate-buffered honey solutions to each of which is added as substrate the same volume of a one per cent soluble starch test solution, the quantity of the former being such that the proportion of the latter progressively decreases in the series with the third tube; (*b*) hydrolysis of the starch for one hour at a temperature of 45°–47°; (*c*) colorimetric determination of the volume of substrate converted by the diastase in one gram of honey, the tube that contains unaltered starch and the highest proportion of honey serving as the basis for the calculations involved.

The suspicion which arose in attempts of the associate referee to repeat these studies, that other factors, apart from the one to which attention has already been directed, might also affect the observed diastatic activity prompted an investigation of the influence exerted upon it by the character of the starch and the quantity of iodine test solution used. Such actually proved to be the case. In carrying out these studies one per cent filtered substrate solutions were made from eight soluble starches. Different degrees of age and different methods of preparing these solutions

from the raw material constitute the variables introduced. Ten, rather than twelve, comparison tubes were used. Five comb honeys of established purity served as experimental material. Full data have been published elsewhere.[1] They are summarized in Table 1.

TABLE 1

Diastase value of honey as affected by character of substrate solution

HONEY	DIASTASE VALUE AS SHOWN BY IODINE TEST SOLUTION ADDED		DESCRIPTION OF STARCH SOLUTION
	1 DROP	2 DROPS	
Clover	39	—	At least 15 years old[a].
	29	39	Fresh starch, solution kept at b.p. of water 0.5 hr.[b]
Buckwheat	23	29	Fresh starch, solution boiled for 3 min.[c]
Tulip-poplar	29	29	See solution *b*.
	14	18	Potato starch boiled for 10 min. with ethyl alcohol containing 0.75% HCl[d]
Alfalfa	39	50	See solution *b*.
	23	23	See solution *d*.
	29	39	See solution *a*.
	29	39	Starch *a* given a preliminary washing with 2% NH₄OH solution, followed by water. Dried before use.[e]
Clover	50	50+	See solution *b*
	29	29	See solution *d*

Limited in scope as these studies may seem, yet it is obvious that the character of the soluble starch which is employed as a substrate, and to some extent the interpretation of the end point of the reaction as influenced by the volume of indicator solution added, are factors which require standardization before this mode of procedure for determining the diastatic activity of honey may find a place in the Association's recognized methods of analyses. Fundamentally the method appears to be sound. Its pressing needs are further refinements.

RECOMMENDATIONS[2]

It is recommended—

(1) That studies on methods for determining the diastatic activity be continued.

(2) That determination of moisture in honey be given critical attention.

[1] Schuette, H. A. and Pauly, R. J., *Ind Eng. Chem Anal. Ed.*, **5**, 53 (1933)
[2] For report of Subcommittee A and action of the Association, see *This Journal*, **16**, 45 (1933).

REPORT ON MAPLE PRODUCTS

By J. F. SNELL (Macdonald College, Province of Quebec, Canada),
Associate Referee

In the collaborative work of 1931 it was found that solutions made from the dry basic lead acetates on the market varied widely in alkalinity and yielded widely varying Canadian lead values in the same sirups and that solutions yielding more concordant results could be obtained by dissolving litharge, activated by heating to 650–670° for 2½–3 hours, in normal lead acetate solution. In order to extend information about this proposed reagent as regards the variability of its lead values in genuine sirups and the effect of adulteration upon such lead values the number of samples distributed to collaborators this year was extended to 40, of which 10 were adulterated.

Instructions for work on several of the other analytical methods were issued, but collaborators were asked to give first attention to the refractometric total solids, the preparation of sample, and the determination of Canadian lead value with the new reagent and that of the Winton lead value with the same reagent. Those equipped for the measurement of electrical conductivity were asked to determine conductivity value and to advise the associate referee regarding revision of the directions for that determination. In Table 1 are given the names and addresses of the collaborators who reported on one or more of the methods and a reference letter consistent with the letters used in previous reports.

The pure sirups, which were donated by the Quebec Department of Agriculture (Hon. Adelard Godbout, Minister), were collected by Les Producteurs de Sucre d'Erable de Québec, a cooperative society of which an official of the Department is secretary. In Tables 2, 3, and 4, respectively, will be found descriptions of the pure sirups, of the mixtures of these used in preparing the adulterated samples, and of the adulterated samples themselves.

REFRACTOMETRIC TOTAL SOLIDS

The attention of collaborators was called to the following possible sources of error in the use of refractometers of the Abbé type:

(1) *Error in setting.*—Correct by use of standard plate or of pure nitrobenzene, methyl salicylate, or benzyl benzoate.

(2) *Inadequate temperature control.*—Circulate water of room temperature through jacket and correct to 20° by use of Stanek's correction table or by addition or subtraction of 0.07 per cent solids for each degree above or below 20°, respectively.

(3) *Dew deposition.*—Eliminated by keeping the prisms at room temperature by means of circulating water.

(4) *Residues from previous measurements on prisms or embedding cement.*—Clean prisms with moist, then with dry cloth or lens paper. Repeat measurements with successive drops of sirup until constant readings are obtained.

TABLE 1

Collaborators on maple products, 1932

REFER-ENCE LETTER	LABORATORY	ADDRESS	DIRECTOR	ANALYST
F	Macdonald College	Macdonald College, P.Q., Canada	J. F. Snell	G. H. Findlay
D	"	"	"	John Duckworth
G	New York Sugar Trade	New York, N.Y.	F. W. Zerban	C. A. Gamble
S	"	"	"	Louis Sattler
Go	Fleischmann	"	C. N. Frey	H. C. Gore
B	Chem. & Tech. Res., U.S. Bur. Chem. & Soils	Washington, D.C.	R. T. Balch	S. Byall
J	Dom. Exptl. Farms System	Ottawa, Can.	F. T. Shutt	A. C. Medcalf (under J. T. Janson)
2W	Name withheld	Washington, D.C.	Names withheld	
H	Acadia Univ.	Wolfville, N.S., Canada	D. U. Hill	D. U. Hill

A fifth source of error is, of course, a blunder in the reading of the scale, in transcribing figures, in converting refractive index to per cent solids, or in applying the temperature correction (adding instead of subtracting, or vice versa). It would appear probable that two extreme results reported, viz. 61.4 for No. 19 in which the other reports were all between 69.2 and 70.1, and 65.8 for No. 38 in which all others were between 62.8 and 63.4, are to be attributed to blunders of this type. Repetitions of the refractometric observations upon some of the sirups, made by two of the collaborators upon portions sent them for the purpose, resulted in all instances in essential confirmation of results that were within the range of those reported by the other collaborators, while results originally extreme were brought within such ranges. In Table 5 both the original and the corrected reports on such sirups are recorded.

Table 6 brings out the fact that four of the seven collaborators who made refractometric observations upon all the samples had a tendency to get high, the remaining three a tendency to get low, results. Such tendency was extreme in the case of one analyst of each group. The one who got the greatest number of minimum results was consistently low, having the

minimum report in 32 samples and the maximum in none. The collaborator who had the largest number of maxima, however, also gave minimum reports in four samples. If the results of these two collaborators were disregarded, there would be no instance of a range of more than 0.8 per cent of total solids in the reports of collaborators and only two of 0.75 per cent or more. Including all results as at first reported there are 28 samples

TABLE 2
Pure maple sirups examined collaboratively in 1932

NO.	FARM NO.	ADDRESS	COUNTY	RUN	COLOR	TOTAL SOLIDS	USED IN MIX-TURE
1	I	Lambton	Frontenac	Middle	Fancy	66.20	A
2	I	Lambton	Frontenac	Early	Light	66.65	A
4	I	Lambton	Frontenac	Middle	Medium	66.47	B
5	I	Lambton	Frontenac	Last	Dark	66.40	C
6	II	Dorset	Frontenac	Middle	Fancy	65.72	A
8	II	Dorset	Frontenac	Early	Light	66.15	—
9	II	Dorset	Frontenac	Late	Medium	67.53	B
10	III	St. Methode	Frontenac	Middle	Light	69.20	—
11	III	St. Methode	Frontenac	Late	Medium	67.23	—
12*	III	St. Methode	Frontenac	Last	Dark	63.89	—
13	IV	St. Paul de Chester	Arthabaska	Early	Light	68.77	A
14	IV	St. Paul de Chester	Arthabaska	Middle	Medium	68.95	—
17	IV	St. Paul de Chester	Arthabaska	Late	Dark	69.32	C
18	V	Warwick	Arthabaska	Middle	Light	67.90	A
19	V	Warwick	Arthabaska	Late	Medium	69.54	—
21	V	Warwick	Arthabaska	Last	Dark	66.84	C
22	VI	Ste. Sophie	Megantic	Middle	Light	66.93	—
23	VI	Ste. Sophie	Megantic	Middle	Medium	62.20	B
24	VI	Ste. Sophie	Megantic	Last	Dark	62.28	—
26	VII	Eastman	Brome	Early	Light	63.71	—
28*	VII	Eastman	Brome	Middle	Fancy	62.32	—
29	VII	Eastman	Brome	Middle	Medium	66.98	D
30	VII	Eastman	Brome	Late	Dark	57.21	C
31	VIII	St. Jean Baptiste	Megantic	Early	Fancy	66.80	A
34	VIII	St. Jean Baptiste	Megantic	Middle	Light	64.45	—
35	VIII	St. Jean Baptiste	Megantic	Late	Medium	68.38	B
36	VIII	St. Jean Baptiste	Megantic	Last	Dark	64.98	C
37	IX	Princeville	Arthabaska	Early	Light	60.88	D
38	IX	Princeville	Arthabaska	Middle	Fancy	62.98	—
40	IX	Princeville	Arthabaska	Middle	Medium	63.45	B

* Fermented.

showing a range of 0.75 per cent or more, 15 with ranges of 1.0 per cent or more, 7 with ranges over 1.25, including, in addition to the two extreme results referred to in the last paragraph, one with a range of 1.6 and four with ranges of 1.3 per cent. It would therefore appear that in ordinary routine, variations of 1.25 to 1.3 per cent among analysts are unavoidable,

but that the most careful workers may hope to reach agreements within 0.75 per cent.

PREPARATION OF SAMPLE

The tentative method[1] was prescribed with omission of the concluding sentence (suggesting the use of hot water funnel, suction or filter aid), but

TABLE 3

Mixtures used in preparing adulterated sirups

(Analyses by Analyst F.)

DESIGNA-TION	PURE SIRUPS REPRESENTED	COLOR	TOTAL SOLIDS	CANADIAN LEAD VALUE*	WINTON LEAD VALUE*	CONDUC-TIVITY VALUE
			per cent			
A	1, 2, 6, 13, 18, 31.	Light	66.70	3.10	1.65	143
B	4, 9, 23, 35, 40.	Medium	65.60	4.50	2.10	158
C	5, 17, 21, 30, 36.	Dark	64.70	5.75	2.46	187
D	29, 37.	Medium	64.50	3.38	1.68	136
	Average			4.18	1.97	156

* F's determination with "activated litharge" reagent.

it was suggested that the instructions to boil to a total solids content of 64.5 per cent before filtering be interpreted to mean that the boiling should be continued to such a total solids content as will yield a *filtered* sirup of 65.0 per cent solids. Whether 64.5 per cent or some other value is most suitable will depend upon variable circumstances. A summary of results is given in Table 7.

TABLE 4

Adulterated sirups examined collaboratively in 1932

NO.	COMPOSITION	CANADIAN LEAD VALUE		WINTON LEAD VALUE		CONDUCTIVITY	
		FOUND (F)	INDICATED MAPLE CONTENT	FOUND (F)	INDICATED MAPLE CONTENT	FOUND (F)	INDICATED MAPLE CONTENT
	per cent per cent		*per cent*		*per cent*		*per cent*
3	80A+20S	2.38	77	1.21	73	127	89
7	65A+35S	1.80	58	0.97	59	101	71
15	50A+50S	1.43*	46*	0.78	47	81	57
16	80B+20S	3.40	76	1.66	79	130	82
20	65B+35S	2.50	56	1.28	61	110	70
25	50B+50S	1.68	37	0.97	46	89	56
27	80C+20S	4.38	76	1.93	79	153	82
32	65C+35S	3.41	59	1.62	66	129	69
33	50C+50S	2.17	38	1.14	46	103	55
39	50D+50S	1.08	32	0.70	42	77	57

* F's Canadian lead value on Sirup No 15 is much higher than those of the other collaborators. Had it been as low as the mean of those (viz. 1.10) the indicated maple content would have been 35.5%, which is comparable with those indicated in the other 50% samples, Nos. 25, 33 and 39.

[1] *Methods of Analysis*, A.O.A.C., 1930, 391, 103(a) 2.

TABLE 5

Refractometric total solids in sirups as received by analysts

SIRUP NO.	ANALYST							REPETITIONS	MEAN*	RANGE	RANGE % MEAN	2W†
	F	G	Go	D	B	J	H					
1	65.94	66.1	66.2	65.8	65.9	66.2	65.95		66.01	0.40	0.61	66.0
2	66.15	66.5	66.8	66.1	66.0	66.5	66.31		66.34	0.80	1.21	66.6
3	65.98	66.4	66.25	66.2	65.9	66.2	66.02		66.14	0.50	0.76	66.6
4	65.94	(66.6)	66.2	66.0	65.8	66.2	65.90	G 66.2	66.03	0.40	0.61	66.5
5	65.84	(66.8)	66.3	66.0	65.9	66.3	66.04	G 66.2	66.08	0.46	0.70	66.5
6	65.55	65.8	65.7	65.4	65.2	65.8	65.20		65.52	0.60	0.92	66.4
7	65.85	66.2	65.8	65.8	65.7	66.1	65.97		65.92	0.50	0.76	66.2
8	65.80	66.2	66.25	65.9	65.5	66.1	65.90		65.95	0.75	1.14	66.3
9	67.45	(66.3)	67.8	67.2	67.3	67.8	67.50	G 67.5	67.51	0.60	0.89	67.9
10	69.18	69.5	69.8	68.8	69.0	69.4	69.02		69.24	1.00	1.44	69.5
11	67.10	67.2	67.5	67.0	66.9	67.2			67.15	0.60	0.89	67.8
12	64.00	64.3	64.2	(64.8)	64.1	64.2		D 64.3	64.18	0.30	0.47	64.4
13	68.90	69.1	69.0	68.9	68.6	69.2			68.95	0.60	0.87	69.0
14	68.95	69.2	69.7	69.0	68.7	69.3			69.14	1.00	1.45	69.6
15	65.74	65.8	66.25	65.5	65.3	65.8			65.73	0.95	1.45	66.6
16	65.52	65.6	65.75	65.7	65.1	65.6			65.55	0.65	0.99	66.4
17	69.50	(70.0)	70.1	69.4	69.2	69.8		G 69.6	69.60	0.90	1.29	70.2
18	67.74	67.9	68.2	(67.0)	67.5	68.0		D 68.0	67.89	0.70	1.03	68.2
19	69.54	70.0	70.1	69.3	69.2	69.6			69.62	0.90	1.29	61.4
20	65.34	65.4	65.5	65.1	65.1	65.4			65.31	0.40	0.61	66.0
21	66.95	67.0	67.2	66.6	66.5	67.0			66.88	0.70	1.05	66.8

22	66.93	67.1	67.2	66.8	66.4	67.0			66.91	0.80	1.20	67.0
23	61.97	(63.1)	62.4	61.8	61.8	62.4		G 62.3	62.11	0.60	0.97	61.9
24	62.27	62.7	62.5	62.1	62.1	62.4			62.35	0.60	0.96	63.1
25	65.07	65.4	65.3	65.1	64.7	65.1			65.11	0.70	1.08	65.7
26	63.78	64.1	64.2	63.8	63.3	64.0			63.86	0.90	1.41	64.6
27	64.58	64.9	65.0	64.7	64.3	64.8			64.71	0.70	1.08	65.0
28	62.47	(62.3)	62.8	62.3	62.2	62.6		G 62.4	62.46	0.60	0.96	63.4
29	67.15	67.4	67.2	66.9	66.8	67.0			67.08	0.60	0.89	66.4
30	57.26	57.4	57.7	57.3	57.0	57.6			57.38	0.70	1.22	58.2
31	66.70	66.9	66.8	66.7	66.4	66.7			66.70	0.50	0.75	67.0
32	64.77	65.1	65.0	64.7	64.5	65.0			64.85	0.60	0.93	65.3
33	64.70	64.9	65.0	65.0	64.4	65.0			64.83	0.60	0.93	64.4
34	64.55	64.7	64.7	64.5	64.3	64.6			64.56	0.40	0.62	65.1
35	68.46	68.7	68.8	68.3	68.3	68.6			68.53	0.50	0.73	69.3
36	65.18	65.5	65.4	65.4	65.0	65.2			65.28	0.50	0.77	65.5
37	60.88	61.0	60.7	69.9	60.5	61.0			60.83	0.50	0.82	61.5
38	62.98	(65.8)	63.2	62.8	62.8	63.4		G 63.1	63.05	0.60	0.95	63.1
39	64.60	64.8	64.8	64.8	64.3	64.6			64.65	0.50	0.77	64.4
40	63.60	(64.6)	64.2	63.5	63.2	63.6		G 63.6	63.62	1.00	1.57	63.0
Av.	65.50	65.8	65.8	65.5	65.3	65.7			65.59	0.64	0.98	65.7

* In calculating the mean, range, and range % mean the values in parentheses were omitted, repetition values being substituted.
† Excluded in calculating mean, range, and range % mean.

Of the analysts, F realized a narrower range in the 40 sirups than last year in 8 sirups and an average total solids nearer to 65.0 per cent. With one exception all the other analysts had average total solids within 0.53 of 65 per cent, though their ranges in the 40 sirups were wider than last year in 8 sirups. Analyst H, who reported on 10 samples, had practically the same average total solids and range as last year. The remaining collaborator obtained results ranging from 64.1 to 73.9, average 67.43. If

TABLE 6

Total solids by refractometer

(Summary of average and extreme results)

ANALYST	AVERAGE TOTAL SOLIDS	EXCLUDING 2W			INCLUDING 2W AND RESULTS OF G AND D REPLACED BY REPETITION		
		NUMBER OF MAXIMUM REPORTS	NUMBER OF MINIMUM REPORTS	TOTAL EXTREME REPORTS	NUMBER OF MAXIMUM REPORTS	NUMBER OF MINIMUM REPORTS	TOTAL EXTREME REPORTS
	per cent						
2W	65.7	—	—	—	26	4	30
Go	65.8	27	0	27	13	0	13
G	65.8	14	0	14	4	0	4
J	65.7	10	0	10	5	0	5
F	65.5	0	2	2	0	2	2
D	65.5	2	8	10	1	8	9
B	65.3	0	35	35	0	32	32
Total		53	45	98	49	46	95

these very erratic results are omitted, the general average of those who reported on all samples is 65.35 per cent and the average range 1.91 per cent. Taken in conjunction with the data of previous reports (see Table 8) these results may be regarded as establishing the advantage of using refractometric control in the preparation of samples.

TABLE 7

Refractometric total solids in samples as prepared for analysis—after filtration but before adjustment

ANALYST	F	G	D	B	J	Av.	2W	H (10 SIRUPS)
Total solids(%)								
Average	65.14	65.53	65.25	65.32	65.50	65.35	67.43	65.24
Maximum	65.45	66.6	65.8	67.6	67.2		73.90	66.16
Minimum	64.68	64.8	64.6	64.5	64.5		64.10	64.58
Range	0.77	1.8	1.2	3.1	2.7	1.91	9.80	1.58

CONDUCTIVITY VALUE

It was recommended that the work on conductivity value for 1932 be directed towards the revision of the directions with special reference to the type of cell and to the determination of the cell constant. Suggestions

made by collaborators S and J, based on experiments made with 0.01 and 0.02 *M* solutions of potassium chloride at 20° and 25° and on the distilled water used, and the experience of analysts F and D in the Macdonald College laboratory led to the revision proposed in the recommendations[1] attached to this report.

The results reported on the 40 sirups are given in Table 9. In the six instances in which the spread between analysts' reports exceeded 5 per cent of the mean, three of the four analysts agreed closely but not always the same three, F being high in sirup A3, J high in A20 and in 23 and D low in A16, A20 and 34. The proportion of samples in which analysts differed by over 5 per cent of the mean and the average range of the analysts' results are considerably less than last year. The average value

TABLE 8
Preparation of sample without and with refractometric control

	WITHOUT	WITH REFRACTOMETRIC CONTROL			
	1929	1930	1931	1932 OMITTING 2W	1932 ALL REPORTS
Number of analysts	10	8	11	6	7
Number of preparations	202	164	88	210	250
Solids of filtered sirup—General Average	61.60	65.60	65.34	65.35	65.68
Lowest average by an analyst	57.4	65.3	64.7	65.1	65.1
Highest average by an analyst	65.3	66.6	66.3	65.5	67.4
Average difference between analysts' maximum and minimum	7.7	2.58	1.35	1.91	3.23
Maximum range for an analyst	11.5	5.47	3.40	3.10	9.80
Minimum range for an analyst	5.8	0.71	0.55	0.77	0.77
Number of analysts preparing all within 5%	0	7	11	6	6
2%	0	3	10	4	4
1%	0	1	6	1	1

for the 30 pure samples, viz. 159, is identical with that found in 24 pure sirups in 1930 with the use of 25 grams of dry matter per 100 cc. and intermediate between the average found in 8 samples in 1931, viz. 179, and that in 20 samples (22 grams dry matter) in 1929, viz. 148. The minimum mean value in a pure sirup was 134, coinciding with that found in the 24 pure sirups examined in 1930. Only one of the 10 adulterated sirups (A27) exceeded this minimum, though all those with less than 50 per cent of adulteration gave values above the minimum previously recorded for solutions containing 22 grams of dry matter per 100 cc.[2]

[1] *This Journal*, **16**, 45 (1933).
[2] Snell, Trans. Roy. Soc. Can. 1919, III, 228.

TABLE 9
Conductivity values

NO.	S	F	D	J*	MEAN	RANGE OF ANALYSTS	RANGE % OF MEAN	DISCORDANT RESULTS
1	135	136	139	136	137	4	2.9	
2	140	141	140	140	140	1	0.7	
A 3	119	127	119	119	121	8	6.6	F high
4	158	158	150	155	155	8	5.2	D low
5	181	182	179	178	180	4	2.2	
6	151	151	152	148	151	4	2.6	
A 7	102	101	102	103	102	2	2.0	
8	147	147	147	147	147	0	0.0	
9	161	160	161	158	160	3	1.9	
10	173	177	170	170	172	7	4.1	
11	189	187	186	187	187	3	1.6	
12	211	206	204	206	207	7	3.4	
13	164	163	160	160	162	4	2.5	
14	154	152	154	154	154	2	1.3	
A15	82	81	83	84	82	3	3.7	
A16	130	130	123	130	128	7	5.5	D low
17	179	178	177	175	177	4	2.3	
18	147	146	147	147	147	1	0.7	
19	158	162	158	157	159	5	3.2	
A20	109	110	111	120	113	11	9.7	J high
21	177	179	177	175	177	4	2.3	
22	149	151	148	149	149	3	2.0	
23	165	165	163	179	168	16	9.5	J high
24	173	178	168	172	173	10	5.8	
A25	88	89	91	92	90	4	4.4	
26	137	137	133	138	136	5	3.7	
A27	150	153	149	149	150	4	2.7	
28	158	160	161	158	159	3	1.9	
29	134	137	134	132	134	5	3.7	
30	215	216	206	213	213	10	4.7	
31	143	145	141	143	143	4	2.8	
A32	128	129	127	130	129	3	2.3	
A33	102	103	103	105	103	3	2.9	
34	148	148	142	150	147	8	5.4	D low
35	145	146	143	147	145	4	2.8	
36	165	166	166	165	166	1	0.6	
37	134	135	132	136	134	4	3.0	
38	136	138	133	138	136	5	3.7	
A39	76	77	76	79	77	3	3.9	
40	161	164	161	148	161	6	3.7	
Average	147	147	145	147	147	5	3.35	
Average in pure sirups	160	160	158	159	159	4.8	3.07	
Maximum in pure sirups	215	216	206	213	213			
Minimum in pure sirups	134	135	132	132	134			
Range in pure sirups	81	81	74	81	79			
Range % Average	51	51	47	51	50			
Range % Minimum	60	60	56	61	59			
Cell constant	.1526	.1362	.1342	.1630				

* J's measurements were made at 20° and calculated to 25° by a factor, 1.13, derived from experience with the 1931 A.O.A.C. samples.

TABLE 10

Canadian lead values with activated litharge reagent—pure sirups

ANALYST	F	D	B	J	H	Hn	D(a)	D(b)	RANGE OF ANALYSTS	GENERAL AVERAGE
No. of sirups	30	30	30	30	8	5	16	14		
Av. lead values	4.42	4.64	5.00	4.45	4.12	4.65	4.78	4.48	2.34	4.57
Max. lead values	7.93	8.27	8.78	8.28	5.86	5.43	6.48	8.27		
Min. lead values	2.61	2.72	2.75	2.18	2.96	3.59	3.11	2.72	0.14	
Range	5.32	5.55	6.03	6.10	2.90	1.84	3.37	5.55		
Range % Av.	120	120	121	137	70	40	71	124		
Range % Min.	204	204	219	280	98	51	109	204		

Analysis of Reagents:*

ANALYST	F	D	B	J	H	Hn	D(a)	D(b)	MEAN	RANGE	RANGE % OF MEAN	Hn†
pH	7.5	See	7.5	7.6	*7.6*	Analysis in Table 7 of 1931 report shows higher Pb and alkalinity than any of the 1932 reagents	7.5	7.5	7.53	0.1	1.33	7.6
Density	1.251	D(a)	1.249	1.250	*1.252*		1.251	1.251	1.251	0.003	0.240	1.255
Alkalinity	9.97	and	9.98	10.05	*10.16*		10.10	9.29	9.925	0.87	8.77	10.51
Total Pb	0.2338	D(b).	0.2316	0.2333	*0.2354*		0.2352	0.2324	0.2336	0.0038	1.63	0.2385
Neutral Pb (N)	0.1305		0.1282	0.1292	0.1301		0.1306	*0.1361*	0.1308	0.0079	5.81	0.1296
Basic Pb (B)	0.1033		0.1034	0.1041	*0.1053*		0.1046	0.0963	0.1028	0.0090	9.35	0.1089
Ratio N:B	1.26		1.24	1.24	1.24		1.25	*1.41*	1.273	0.17	13.35	1.19

* All analyses by F
† From 1931 Report.

CANADIAN LEAD VALUE

Determinations of Canadian lead value with the use of the activated litharge reagent (Table 10) were made by four analysts on all the sirups and by a fifth (H) on Nos. 1–10. On Nos. 6–10, Analyst H also repeated his determinations, using the activated litharge reagent he had prepared in 1931.[1] As analyzed in 1931 this reagent was a little denser than any of the others of either 1931 or 1932. Its total lead content and its alkalinity were also greater than those of any of the other reagents. The amount of reagent (designated a) prepared by analyst D at the outset proved to be insufficient for all the analyses, and a second reagent (b) had to be prepared for the analysis of sirups Nos. 17–22, 24–27, 29–37 and 39. Reagent (b) was prepared from the litharge activated a fortnight previously for preparation of reagent (a). Upon analysis it was found to have much lower alkalinity, and accordingly a higher ratio of neutral to basic lead,

TABLE 11

Canadian lead values with reagents prepared from dry basic lead acetate pure sirups

ANALYST	F	B
Number of sirups	4	30
Average lead value	4.19	3.79
Maximum	5.62	6.03
Minimum	3.32	2.27
Range	2.40	3.76
Range % Average	57	99
Range % Minimum	75	166
Analysis of reagents:		
pH	7.4	7.1
Density	1.250	1.247
Alkalinity	8.91	5.02
Total Pb	0.2352	0.2236
Neutral Pb (N)	0.1429	0.1716
Basic Pb (B)	0.0923	0.0520
Ratio N:B	1.55	3.30

than any other of either 1931 or 1932. With the exception of this one the reagents prepared in 1932 show remarkable similarity. This may, of course, be fortuitous, but it raises the question of how long the activation of the litharge persists.

When all six of the 1932 reagents are compared, the variations between reagents are found to be smaller as regards specific gravity, total lead, and neutral lead, but greater as regards alkalinity and basic lead than those in the nine reagents analyzed in 1931. As in 1931, all the reagents prepared in this way have pH values of either 7.5 or 7.6, corresponding to a range of 22 per cent in concentration of hydrogen ion.

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[1] See Table 7 of 1931 report. *This Journal*, 15, 190 (1932).

In spite of the close similarity of the reagents of analysts F, D(a), B, J and H, the lead values given by them in identical sirups show wide variations. The average range in the collaborators' lead values on the pure sirups is 17.5 per cent of the mean. In individual sirups the range runs from 5 to 47 per cent of the mean. In the adulterated sirups this range averages 18.6 per cent and varies in the individual sirups between 7 and 45 per cent of the mean. It is also remarkable that the reagent D(b), though differing from the others so notably as regards analysis, gave no lead value outside the range of those yielded by the other reagents. The reason for the wide differences among collaborators is not clear.

Analysts B and F prepared reagents from dry basic lead acetate. These were found to be decidedly less alkaline than those made from the activated litharge. The two were also quite dissimilar in lead content and in alkalinity (see Table 11). B determined lead values on all the sirups. F used his reagent only in a study of the effect of adulteration on the lead value, using as pure sirups the mixtures A–D described in Table 3. Except in two of the adulterated samples, B always obtained lower lead values with this reagent than with that prepared from activated litharge. F's average for the four mixed pure sirups is the same for both reagents.

The range of variation of the lead values in pure sirups appears to be narrower with the reagent prepared from the basic lead acetate than from that prepared from activated litharge, being in B's work 99 per cent of the average for the former reagent and 121 per cent for the latter. In view of the great variability of solutions prepared from commercial samples of dry basic lead acetate, however, it would not be safe to assume that such solutions have an advantage in respect to variability of lead number in genuine sirups over those made from activated litharge. The evidence is too meager to justify a definite conclusion on this point.

In Table 12 the effect of adulteration of maple sirup with refined sugar upon the Canadian lead values is illustrated with reference to the two forms of the reagent. It will be noted that the indicated maple content of the adulterated sirups is very nearly the same for both, being always below, and in the most highly adulterated samples very far below, the actual maple content. These experiments on four genuine sirups, showing a fairly wide range of lead value, indicate that no loss in delicacy would be involved in substituting the activated litharge reagent for that now in common use.

WINTON LEAD VALUES

Winton lead values were determined on all 40 sirups by five collaborators using the reagent from activated litharge after the dilution with four volumes of water prescribed in the directions. One collaborator (B) determined this value also with reagents made, respectively, from unactivated litharge and from dry basic lead acetate. Results are given in Table 13. The solutions prepared from unactivated and activated litharge

were very similar and gave nearly equal lead values, though the minimum value found in the pure sirups was materially lower with the reagent made from the unactivated oxide. That made from dry basic lead acetate was much less alkaline than the others and gave lower lead values in all the sirups except No. 35. On the average the Winton lead values of the pure sirups are 32 per cent higher with the activated litharge than with the dry basic lead acetate. The basic lead acetate solution, however, shows the narrowest range among the pure sirups, being 66 per cent of the average and 94 per cent of the minimum, as against 85 per cent and 133 per cent for the activated and 91 per cent and 157 per cent for the unactivated litharge. The agreement of analysts is much closer on the Winton than on

TABLE 12

Comparison of reagents as regards falling off of lead values in
progressive adulteration of sirups

SIRUP NO.	ACTUAL MAPLE CONTENT	DRY BASIC ACETATE REAGENT		ACTIVATED LITHARGE REAGENT	
		LEAD VALUE	INDICATED MAPLE CONTENT	LEAD VALUE	INDICATED MAPLE CONTENT
	per cent		*per cent*		*per cent*
A	100	3.32		3.10	
3	80	2.15	67	2.38	77
7	65	1.96	61	1.80	58
15	50	1.05	33	1.43	46*
B	100	4.48		4.50	
16	80	3.40	76	3.40	76
20	65	2.59	58	2.50	56
25	50	1.75	39	1.68	37
C	100	5.62		5.75	
27	80	4.40	78	4.38	76
32	65	3.50	62	3.41	59
33	50	2.26	40	2.17	38
D	100	3.43		3.38	
39	50	1.18	34	1.08	32

* 35.5 if mean of other collaborators' lead values for sirup No. 15 be substituted for F's (see footnote to Table 4).

the Canadian lead values. The redissolving effect of the excess of basic lead acetate would naturally be much less in the concentrations represented in the Winton than in those involved in the Canadian method. This suggests that in the Canadian method errors in the measurement of the small volume of reagent (2 cc.) used may be responsible for much of the discordance among collaborators.

In Table 14, the actual maple content of each of the adulterated sirups is compared with that indicated by the Winton lead value. In general the indicated is a little below the actual content. Some, but not all, of the

TABLE 13

Winton lead values

ANALYST	REAGENT MADE FROM:											
	NORMAL ACETATE AND LITHARGE								UNACTIVATED	DRY BASIC ACETATE		
	ACTIVATED					MEAN	RANGE OF ANALYSTS	RANGE % OF MEAN				
	F	D	B	J	2W				B	B	DIFF.*	DIFF. % BASIC
Av. in pure sirups	2.01	2.00	1.95	2.02	1.91	1.89	0.20	10.6	1.98	1.48	0.47	32
Max. in pure sirups	2.95	2.88	2.91	3.08	2.87	—	0.36	23.7	2.96	2.02	0.99	—
Min. in pure sirups	1.37	1.30	1.25	1.31	1.23	—	0.07	3.1	1.15	1.04	−0.08	109
Range in pure sirups	1.58	1.58	1.66	1.77	1.64	—	0.29	20.6	1.81	0.98	1.07	—
Range % Av.	79	79	85	88	86	—	145		91	66	108	
Range % min.	115	122	133	135	133	—	414		157	94	—	
Reagents before dilution:												
pH	See	See	7.5	See	Not				7.5	7.1		
Density	Table	Table 10	1.249	Table 10	analyzed				1.251	1.247		
Alkalinity	10	D(a)	9.98						9.93	5.02		
Total Pb		and	0.2316						0.2334	0.2236		
Neutral Pb (N)		D(b)	0.1282						0.1305	0.1716		
Basic Pb (B)			0.1034						0.1029	0.0520		
Ratio N:B			1.24						1.27	3.30		

* Difference between the lead numbers determined with the activated litharge and dry basic acetate solutions

sirups similarly adulterated in 1913[1] also gave low indications but the falling off of the value with increasing adulteration was not so great as in the Canadian lead number.

There appears to be no reason why the Winton reagent should not be made by dilution of that made from activated litharge for use in the Canadian method, but as in the latter method, higher lead values are to be expected than were yielded by the reagent made from at least some brands of dry basic acetate.

SUMMARY

1. Results of work by 9 collaborators in 7 laboratories on 30 pure and 10 adulterated maple sirups are reported.

TABLE 14
Winton lead values (F's analyses). Effect of adulteration

SIRUP NO.	LEAD CONTENT	MAPLE CONTENT	
		ACTUAL	INDICATED
		per cent	*per cent*
A	1.65	100	—
3	1.205	80	73
7	0.97	65	59
15	0.78	50	47
B	2.095	100	—
16	1.655	80	79
20	1.28	65	61
25	0.97	50	46
C	2.455	100	—
27	1.93	80	79
32	1.62	65	66
33	1.135	50	46
D	1.68	100	—
39	0.70	50	42

2. In the refractometric determination of solids the average range between the maximum and minimum reports of 6 analysts on 40 and one analyst on 10 sirups (250 determinations) was 0.64 per cent of solids. This range is 0.98 per cent of the average of the mean values on the individual sirups, viz. 65.59 per cent solids.

3. The greatest difference between any of these analysts on an individual sirup was 1.00 per cent of solids (or 1.57 per cent of the mean result on that sirup, viz. 63.62 per cent solids). In this and the preceding paragraph of the summary, the very erratic work of one collaborator and 10 results of two other collaborators, which were altered upon repetition of the measurement, are excluded from consideration.

[1] Snell and Scott, *J. Ind. Eng. Chem.* **5**, 993 (1913).

4. In the preparation of sample by reboiling with thermometric and refractometric control, satisfactory results were obtained by all the collaborators except the one whose report on solids was so erratic. One analyst prepared all 40 sirups to within a range of 0.77 per cent solids and 4 of the 7 prepared all within 2 per cent of solids. The maximum range for an analyst was 3.10 per cent solids, which is a little narrower than was realized in 1931 by any of 11 analysts, each preparing 8 sirups. The average difference between the maximum and minimum of each analyst was higher than in 1931 but not so high as in 1930, all three years showing a marked improvement over 1929, when no refractometric control was employed.

5. In conductivity value the average difference between the maximum and minimum reports of analysts was 4.8 units = 3.07 per cent of the average of the means on individual sirups (159). The lowest conductivity reported by an analyst in a pure sirup was 132 and the highest 216. The average for the 30 pure sirups was 159.

6. As in 1932, basic lead acetate solutions prepared from activated litharge and normal lead acetate were found more uniform than those made from commercial dry lead "subacetate."

7. The Canadian lead values, reported by 5 analysts using the activated litharge reagent, show variations ranging from 5 to 47 per cent of the mean, the average range being 17.5 per cent of the mean.

8. In Winton lead values determined with activated litharge reagent collaborators concord much more closely.

9. In the sirups adulterated with refined sugar the Canadian lead values tend to fall off more rapidly than the actual maple content. The Winton lead values exhibit the same tendency to a less marked extent. The conductivity value falls off less rapidly than the maple content.

The recommendations of the associate referee regarding changes in methods were approved. They have been published.[1]

REPORT ON DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS

By Carl F. Snyder (U. S. Bureau of Standards, Washington, D. C.), *Associate Referee*

A. Refractometric Methods.—It was pointed out in the report presented to this Association last year that the arbitrary scale of immersion refractometers of domestic manufacture differed by an appreciable amount from the scale of the Zeiss refractometer. In collaboration with the Committee for a Standard Refractometer Scale a detailed study of the whole subject was made. It is noted that most of the published work based on

[1] *This Journal*, 16, 45 (1933).

data obtained with the immersion refractometer has been confined to the first dipping prism having a range corresponding to refractive indices between 1.32 and 1.36. In order to eliminate the existing uncertainty arising from the two different scales, a conversion table was constructed to give the equivalent scale readings and refractive indices. A full discussion of the subject is given in the report of the committee.[1]

J. F. Snell, the Associate Referee on Maple Products, has called attention to the difficulties encountered in refractometric determinations on the Abbé refractometer by the official method when the readings are made in a humid atmosphere at high temperatures. He suggests that under such conditions the determination be made at room temperature and the reading corrected to the standard temperature by means of Table 7, XLII.[2] It is therefore recommended that the refractometer method[3] be amended by the addition of the following:

If the determination is made at temperatures appreciably above 20° C. or if the humidity causes condensation of moisture on the exposed faces of the prisms, make the measurement at room temperature and correct the readings to the standard temperature of 20° C. by means of Table 7, XLII.

B. Densimetric Methods.—The density work for the year consisted of the determination of the weight per gallon of extracted honey. The associate referee was fortunate in having the collaboration of G. E. Marvin of the Bureau of Entomology and L. D. Hammond of the Bureau of Standards.

The method usually employed for trade purposes in the determination of weight per gallon of honey is to weigh the material contained in a standard measure of convenient size, such as one-fourth or one-half pint. A comparison was made of the weights obtained by this method and the weights per gallon computed from the specific gravities obtained by the pycnometer method described in last year's report.[4] In addition, the refractive indices of the samples were determined on a Spencer-Abbé refractometer.

The specific gravities (20°/4° C.) were converted to weights per gallon in air by means of the Table of Weights Per Gallon of Sucrose Solutions (Table 1, Bureau of Standards Circular No. 375). For convenience of conversion this table was expanded to intervals of 0.1° Brix for the range 65 to 85 Brix and a column was added showing the equivalent refractive indices from the Schönrock-Main table.

In the determination of the refractive indices, the prisms of the refractometer were maintained at 20° C. by circulating water at that temperature from a large water thermostat.

[1] *This Journal* 16, 85 (1933).
[2] *Methods of Analysis A.O.A.C.*, 1930, 512.
[3] *Ibid.*, 365 7.
[4] *This Journal*, 15, 195 (1932).

Extreme care was exercised in cleaning and drying the prisms of the instrument before charging with honey. The circulation of water was continued for a sufficient time to insure temperature equilibrium before readings were made. The instrument was checked with distilled water before and after each series of observations. All work was conducted in a constant temperature room maintained at 20° C. ± 1° C.

The refractive indices were converted to the equivalents in Brix, which in turn were converted to weights per gallon by means of the expanded table mentioned previously. The results of the collaborative work on the 18 honeys are given in the following table:

Weights per gallon of honeys

SAMPLE NO.	BY DIRECT WEIGHING	BY PYCNOMETER	BY REFRACTOMETER	SAMPLE NO.	BY DIRECT WEIGHING	BY PYCNOMETER	BY REFRACTOMETER
	pounds	*pounds*	*pounds*		*pounds*	*pounds*	*pounds*
A	11.993	12.001	11.989	1131	11.909	11.896	11.896
B	11.781	11.791	11.791	1132	11.943	11.955	11.942
C	11.852	11.866	11.839	1133	11.816	11.809	11.793
D	11.852	11.855	11.856	1134	11.816	11.802	11.797
E	11.958	11.957	11.951	1135	11.802	11.798	11.779
F	11.831	11.840	11.834	1136	12.085	12.056	12.048
G	11.802	11.816	11.791	1137	11.746	11.744	11.747
H	11.831	11.825	11.823	1138	11.711	11.717	11.721
1130	11.894	11.911	11.900	1139	11.990	11.966	11.964

The coefficient of cubical expansion was determined for five samples of honey, the average of the values obtained being 0.00047 per unit volume per degree Centigrade. The effect of temperature on the refractive index was measured. The change in index with change of temperature $\left(\dfrac{\Delta n}{\Delta t°}\right)$ was found to be 0.00022.

REPORT ON POLARISCOPIC METHODS

By S. BYALL (Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.) *Associate Referee*

In accordance with the recommendations approved at the last meeting of the Association, the associate referee began a study of methods for preparing invertase whereby the process might be simplified and made more efficient. It was considered advisable to make a survey of the available commercial preparations to determine their suitability for analytical work, as apparently the principal drawback to the invertase method has been the trouble of preparing a suitable purified and standardized solution.

TABLE 1

Sucrose in sugar products determined by commercial invertase

(Results expressed in percentage)

COLLABORATOR	PRODUCT ANALYZED	I	II	III	IV	V	VI
				INVERTASE PREPARATIONS			
Gamble	Sucrose	99.78*	99.92	100.00	100.09	100.04	99.89
Mull	(Domino	100.07	99.92	99.84	100.15	100.12	100.07
Byall	Cube)	100.00	99.93	99.92	100.00	99.92	100.00
	Av.	100.04	99.92	99.92	100.08	100.03	99.99
Gamble	Raw Sugar	98.12	97.85	98.15	98.19	98.15	98.07
Mull		98.45	97.77*	98.33	98.53	98.53	98.45
Byall		98.38	98.41	98.47	98.65	98.59	98.63
	Av.	98.32	98.13	98.32	98.46	98.42	98.38
Gamble	Molasses	37.35	36.97	37.51	37.47	37.43	37.28
Mull		37.17	36.76	37.20	37.25	37.25	37.22
Byall		37.05	36.93	36.93	37.02	37.01	37.01
	Av.	37.19	36.89	37.21	37.25	37.23	37.17

* Cloudy and difficult to read. Not included in average.

Five commercial preparations manufactured by four different companies were compared with an invertase solution made by the official method, which is represented by No. 1 in Table 2; Nos. 2 to 5, inclusive, were glycerin solutions, and No. 6 was a dry preparation of invertase. All these preparations were diluted previous to use to an activity of approximately $K = 0.1$ with water in the case of 1 and 6, and with glycerin (approximately 65 per cent) in the case of Nos. 2 to 5, inclusive. The original activity of these preparations is given in Table 2.

TABLE 2

Original activity of commercial invertase preparation

PREPARATION NO.	K VALUE (20°C.)
1	0.16
2	0.23
3	0.24
4	0.22
5	0.23
6	Dry, not tested

At the suggestion of F. W. Zerban these solutions were tested on impure products, namely, raw sugar and molasses in addition to sucrose (Domino Cube), because he had found that some invertase preparations sometimes failed completely to invert sucrose in the presence of the encountered impurities.

The results of the sucrose determinations on the various products analyzed with the aid of the six invertase preparations are given in Table 1. These data represent the average of two or more separate determinations made by each collaborator. It may be noted that invertase No. 2 tends to yield low results in every case, while the other preparations appear to yield results within experimental error compared to those obtained by No. 1.

Before recommending an approval by the Association of any of the commercial preparations, the associate referee suggests that this work be duplicated next year on other samples of invertase from the same companies.

As a phase of this work it is also planned, with the cooperation of the Food Research Division, to make a study of the invertase content of different strains of yeast in an attempt to find one more suitable than the one generally used for this purpose.

As the work contemplated for next year is covered by recommendations already approved by the Association,[1] no others will be suggested at this meeting.

No report on chemical methods for reducing sugars was given by the associate referee.

REPORT ON FEEDING STUFFS

By V. E. MUNSEY (U. S. Food and Drug Administration,
Washington, D. C.), *Referee*

The Referee on Feeding Stuffs recommends:[2]

(1) That the tentative method for the determination of lime in mineral feeds be further studied.

(2) That work on the comparison of the Knapheide and Lamb method with other methods for iodine be continued.

(3) That the methods for the determination of iodine in organic materials be further studied.

(4) That the study on fat solvents for the determination of fat in feeding stuffs be continued.

(5) That the method for vitamin D assay reported by the associate referee be modified and studied collaboratively.

(6) That any method which may be suggested for cod liver oil detection in mixed feeds be studied.

(7) That a study of the quantitative determination of sodium chloride in feeding stuffs be made.

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[1] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 46 (1933).
[2] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 43 (1933).

(8) That a collaborative study be made of the tentative methods for the determination of hydrocyanic acid formed by the hydrolysis of glucoside-bearing material.

No report on stock feed adulteration was given by the associate referee.

REPORT ON MINERAL MIXED FEEDS

By H. A. HALVORSON (Department of Agriculture, Dairy, and Food, St. Paul, Minn.), *Associate Referee*

Three recommendations for future study were made last year. The work during the current year was confined to the second and third recommendations, which provided that the comparison of the Knapheide and Lamb method[1] with other methods for the determination of iodine be continued and that methods for the determination of iodine in organic substances be further studied. Instructions for the work and two samples were sent, during the past summer, to twenty-five chemists who had expressed a desire to collaborate. Both of the products selected for testing were ground dried kelp or kelp meal. Sample No. 1 was said to be kelp from the Pacific Ocean, obtained off the coast of California. Sample No. 2 was represented as made from kelp harvested near the coast of Alaska.

Collaborators were instructed to determine iodine in both samples by the method of Knapheide and Lamb. The directions specified that the entire sample be ground to pass a sieve of about 30-mesh and that the quantity of the sample taken for analysis be reduced to 2 grams. Attention was also called to the precautions and additional details which had been published in previous reports of the associate referee or included in the instructions sent out in former years. Nineteen collaborators responded with results of analysis obtained by this method. The results of individual determinations and averages are given in Table 1.

While most of the collaborators have calculated and reported their results to the fourth decimal place, a review of all the reports seems to indicate that no significance can be attached to the figures beyond the third decimal place in the case of sample No. 1, and beyond the second decimal place in the case of sample No. 2. The averages reported by a majority of the collaborators on sample No. 1 agree within ±0.005 per cent of the mean. The mean of the twenty averages on sample No. 1 shown in Table 1 is 0.138 per cent, and the averages used for calculation of this mean are recorded to only three places. Fifty-five per cent of the averages reported are within ±0.005 per cent of the mean of the averages; 90 per cent are within ±0.010 per cent; and 95 per cent are within ±0.015 per cent.

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[1] *J. Am. Chem. Soc.*, **50**, 2121 (1928).

COLLABORATORS	SAMPLE NO. 1		SAMPLE NO. 2	
	INDIVIDUAL	AVERAGE	INDIVIDUAL	AVERAGE
E. M. Bailey and W. T. Mathis	0.1394		0.2668	
New Haven, Conn.	0.1363		0.2671	
	0.1394	0.1386	0.2663	0.2662
	0.1394		0.2646	
J. W. Bowen and J. Drain	0.1463		0.2550	
Purina Mills	0.1408		0.2511	
St. Louis, Mo.	0.1410		0.2561	
	0.1409		0.2553	
	0.1447	0.1414	0.2354	0.2478
	0.1411		0.2389	
	0.1380		0.2473	
	0.1382		0.2433	
A. E. Christopher,	0.1325		0.2601	
Research Dept. Kelco Co.	0.1341	0.1346	0.2623	0.2621
Chicago, Ill.	0.1371		0.2640	
G. G. Frary	0.1423		0.2519	
Vermilion, S. D.	0.1468	0.1460	0.2678	0.2613
	0.1488		0.2643	
G. G. Frary and M. H. Shennum	0.1449	0.1449		
Vermilion, S. D.				
G. S. Fraps and T. L. Ogier	0.136		0.167	
College Station, Texas	0.135	0.1355	0.165	0.166
W. C. Geagley	0.143		0.268	
Lansing, Mich.	0.149	0.146	0.261	0.2645
W. B. Griem and L. Clifcorn				
Madison, Wis.				
1 gram sample	0.1424		0.2635	
	0.1451	0.1429	0.2635	0.2637
	0.1411		0.2641	
2 gram sample	0.1415		0.2698	
	0.1335		0.2467	
	0.1300	0.1359	0.2530	0.2583
	0.1378		0.2638	
	0.1367			
W. F. Hand, H. Solomon and S. J.	0.1492		0.2815	
Few	0.1481	0.1469	0.2746	0.2781
A. & M. College, Miss.	0.1434		0.2783	

TABLE 1 (*Continued*)

COLLABORATORS	SAMPLE NO. 1		SAMPLE NO. 2	
	INDIVIDUAL	AVERAGE	INDIVIDUAL	AVERAGE
A. P. Kerr Baton Rouge, La.	0.145	0.145	0.247	0.247
Kraybill and Yung Lafayette, Ind.	0.1407		0.2504	
	0.1404		0.2477	
	0.1392		0.2472	
	0.1383		0.2458	
	0.1373	0.1376	0.2330	0.2354
	0.1368		0.2255	
	0.1344		0.2187	
	0.1334		0.2147	
C. S. Ladd and W. A. Groves Bismarck, N. D.	0.1333		0.2398	
	0.1340		0.2432	
	0.1384		0.2611	
	0.1391	0.1407	0.2616	0.2580
	0.1447		0.2643	
	0.1458		0.2645	
	0.1455		0.2648	
	0.1450		0.2645	
H. W. Loy, Jr. Manhattan, Kan.	0.1188		0.2121	
	0.1176	0.1178	0.2081	0.2100
	0.1170		0.2098	
V. E. Munsey Washington, D. C.	0.1450		0.2694	
	0.1413	0.1432	0.2661	0.2667
			0.2647	
R. C. Newton Swift & Co. Chicago, Ill. Analyst No. 1	0.1282		0.2305	
	0.1312	0.1312	0.2226	0.2231
	0.1342		0.2162	
Analyst No. 2	0.1360		0.2187	
	0.1370	0.1370	0.2258	0.2239
	0.1380		0.2031	
			0.2481	
A. O. Olson St. Paul, Minn. 1 gram sample			0.2386	
			0.2446	
			0.2348	0.2397
			0.2353	
			0.2451	

TABLE 1 (*Continued*)

COLLABORATORS	SAMPLE NO. 1		SAMPLE NO. 2	
	INDIVIDUAL	AVERAGE	INDIVIDUAL	AVERAGE
2 gram sample	0.1378		0.2452	
	0.1300		0.2509	
	0.1266	0.1329	0.2486	0.2475
	0.1373		0.2486	
			0.2430	
			0.2469	
			0.2509	
			0.2458	
E. L. Redfern and R. C. Meehan	0.1457		0.2914	
Des Moines, Iowa	0.1545	0.1501	0.3039	0.2976
H. D. Spears	0.1224		0.2238	
Lexington, Ky.	0.1129		0.2246	
	0.1168	0.1184	0.2229	0.2232
	0.1214		0.2216	

Treating the averages reported on sample No. 2 in a manner similar to the above, it is found that their agreement is not nearly so satisfactory as the results on sample No. 1. Whether or not this can be accounted for by the physical condition of the sample is difficult to say. This product did not have, in its original state, a uniform appearance. So far as even texture and fineness were concerned, it was unlike sample No. 1 and seemed to have a tendency to segregate. While the iodine content of sample No. 2 is nearly twice that of sample No. 1, this fact does not entirely account for the less satisfactory agreement of results obtained. Two of the collaborators, when determining iodine in this product, used a 1 gram sample as well as the specified 2 grams. A 1 gram portion of sample No. 2 gave approximately the same quantity of iodine in the fusion mixture as did 2 grams of sample No. 1. One of the collaborators pointed out that higher results were obtained on both products with 1 gram than with 2 gram portions. Another collaborator reported a lower average result on sample No. 2 after the substitution of a 1 gram portion for the 2 grams.

If the fourth decimal place in the reported averages on sample No. 2 is eliminated, the difference between high and low results is 0.132 per cent, which is about 50 per cent of the apparent amount of iodine in the product. When the highest and lowest averages reported are omitted, it is found that the difference between the second highest and second lowest average is 0.068 per cent. With these omissions, the mean of the 18 remaining averages is 0.243 per cent. Seventeen per cent of the averages reported agree within ±0.005 per cent of this mean, 28 per cent within ±0.010 per cent, 39 per cent within ±0.015 per cent, 67 per cent within ±0.020 per cent, and 89 per cent within ±0.025 per cent.

Collaborator G. E. Grattan, Ottawa, Canada, also made determinations of iodine by the Knapheide and Lamb method, and reported six results on sample No. 1 and five results on sample No. 2. These arrived too late for inclusion in the tabulation. His averages were 0.1432 per cent for sample No. 1 and 0.2662 per cent for sample No. 2.

IODINE IN MINERAL FEED SAMPLES BY OTHER METHODS

This year's instructions contained the additional recommendation that iodine be determined in the two samples by other methods with which the collaborators were familiar. The request was also made that collaborators, in a position to do so, determine iodine in the samples by the micro modification of the McClendon combustion method.[1]

Three chemists made determinations on the two samples by the last-named method. The results are shown in Table 2. The mean of the reported averages (omitting the fourth decimal place), on sample No. 1, is 0.122 per cent. This is 0.016 per cent lower than the mean of the averages obtained by the Knapheide and Lamb method. The mean of the three averages reported on sample No. 2 is 0.208 per cent, and this is found to be 0.035 per cent lower than the accepted average for this sample by the Knapheide and Lamb method. It is apparent that the micro modification of the McClendon combustion method gave lower results than did the Knapheide and Lamb method on the two samples tested this year. In the limited number of results reported, however, the differences between high and low on both samples were considerably less by the McClendon method than by the Knapheide and Lamb method.

As will be seen from Table 2, only one collaborator reported determinations by a method other than the Knapheide and Lamb or the McClendon combustion methods. These results were obtained by a method in use in the laboratories of the Kelco Co. Briefly, the method consists of conducting a fusion of the sample with lime and soda lime, the fused mass being later ground, and boiled with distilled water and filtered. The filtrate, which contains the iodine, is acidulated with dilute sulfuric acid and saturated with nitric oxide. In a separatory funnel, the released iodine is extracted with carbon bisulfide as many times as necessary. The combined portions of carbon bisulfide are washed to free from acid and titrated to a colorless end point in a separatory funnel with a standard sodium thiosulfate solution. The average reported by this method on sample No. 1 agrees very well with the mean of the averages reported by the 19 collaborators using the Knapheide and Lamb method. On sample No. 2 the average reported is 0.021 per cent higher than the accepted mean of the averages by the Knapheide and Lamb method.

It is worth pointing out that the averages obtained by the three dif-

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[1] *J. Am. Chem. Soc.*, **52**, 980 (1930).

ferent methods on sample No. 1 are in much closer agreement than are the corresponding results obtained on sample No. 2. It might also be said that while the results reported by the method of the Kelco Co. are in better agreement with the averages from the Knapheide and Lamb method than with results from the McClendon method, this new procedure has not yet been tried out thoroughly on complex mineral mixtures.

TABLE 2

Iodine in mineral feed samples by other methods than the Knapheide-Lamb method
(Results expressed in percentage)

COLLABORATORS	SAMPLE NO. 1		SAMPLE NO. 2	
	INDIVIDUAL	AVERAGE	INDIVIDUAL	AVERAGE
A. E. Christopher	0.1347		0.2627	
Research Dept. Kelco Co.	0.1347	0.1349	0.2644	0.2644
Chicago, Ill.	0.1353		0.2662	
Kelco Co. Method				
C. S. Ladd and W. A. Groves	0.1315		0.2113	
Bismarck, N. D.	0.1306		0.2118	
Micro modification of the	0.1185		0.2032	
McClendon Combustion	0.1238	0.1266	0.2103	0.2100
Method	0.1259		0.2122	
	0.1250		0.2112	
	0.1289			
	0.1290			
R. E. Remington and E. J. Coulson	0.1245		0.2102	
Charleston, S. C.	0.1183		0.2148	
Micro modification of the	0.1195	0.1188	0.2023	0.2101
McClendon Combustion	0.1186		0.2130	
Method	0.1133			
R. E. Remington and Harry von	0.1268		0.2050	
Kolnitz	0.1124	0.1196	0.2000	0.2025
Charleston, S. C.				
Micro modification of the				
McClendon Combustion				
Method				

The conclusion that can be properly drawn from the results this year is that the Knapheide and Lamb procedure still gives the greatest promise of becoming the accepted method, but this is true partly because more collaborative testing has been done with this method than with the other methods. Further work needs to be done, however, before any method is adopted. It is the opinion of the associate referee that the recommendations made last year should be repeated.

RECOMMENDATIONS[1]

It is recommended—

(1) That the tentative method for the determination of lime in mineral feeds be further studied.

(2) That work on the comparison of the Knapheide and Lamb method with other methods for iodine be continued.

(3) That the methods for the determination of iodine in organic substances be further studied.

No report on moisture in feeding stuffs was given by the associate referee.

REPORT ON BIOLOGICAL METHODS FOR DETERMINATION OF COD LIVER OIL IN FEED MIXTURES

By W. B. GRIEM (Department of Agriculture and Markets, Madison, Wis.), *Associate Referee*

At the last meeting of this Association experimental work was presented[2] and a method was suggested by the associate referee for estimating the vitamin D content of materials used in supplying added vitamin D to feeding stuffs. The associate referee believed that it was most essential that such a method be established in order to advance related work. He was instructed to inaugurate collaborative work. Such assay work is tedious, costly, and more or less inconvenient, as special equipment is necessary and arrangements must be made for birds and supplies. Only one member would have been able conveniently to do collaborative work although several others expressed their willingness had conditions been satisfactory. No collaborative samples, therefore, were sent out, but the associate referee believes that if collaborative study is started about six months before a report is due, the work can be carried out successfully.

No work was conducted according to the additional instructions to study any methods which might be suggested for cod liver oil detection in mixed feeds. No such method was suggested, nor did any that seemed to be of any value occur to the associate referee.

Assay work was continued along the lines reported last year. The useful results obtained in the laboratories of the associate referee when the methods proposed last year were used, and the need for a standardized method for assaying vitamin D supplements for feeding stuffs, seem to justify the recommendation that the method included in this report be adopted as a tentative method. In addition, several feed control laboratories are using the method successfully. It is also used in research work at the University of Wisconsin and by several manufacturers who supply vitamin D carriers to the trade. The method follows:

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[1] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 43 (1933).
[2] *This Journal*, 15, 222 (1932).

VITAMIN D ASSAY BY PREVENTIVE BIOLOGICAL TEST

(Applicable to fish and fish liver oils and their extracts and to materials used for supplementing the vitamin D content of feeds. Not applicable to irradiated ergosterol products or to irradiated yeast unless recommended for poultry.)

BASAL RACHITIC RATION

59% ground yellow corn, 25% pure flour middlings, 12% casein, 1% calcium carbonate (precipitated), 1% calcium phosphate (precipitated), 1% salt, 1% yeast foam tablet (powder, 50% protein).

Place groups of six or more one-day-old white leghorn chickens in screen-bottomed biological cages, out of direct sunlight. Always keep distilled water before them. Reserve one group for negative control purposes, and one or more additional groups for each material to be assayed.

Prepare sufficient basal rachitic ration for the entire feeding period (120 pounds per 100 birds is ample). Prepare the supplemental ration at 12-day periods. Supplement the basal rachitic ration with corn oil in a quantity equal to the maximum addition of the oil to be assayed. (This is the ration to be fed to the negative group.) Supplement the basal ration with different levels of the material to be assayed. Add corn oil to bring the percentage of oil up to that added to the negative control ration. (These are the rations to be fed to the other groups.) (Additions of $\frac{1}{8}$, $\frac{1}{4}$, and $\frac{1}{2}$ per cent are recommended for cod liver oil in order to obtain comparative values. Oils fortified in vitamin D should be added to the basal rachitic ration at lower levels. Materials less potent in vitamin D than cod liver oil should be added to the basal rachitic ration at higher levels.)

On the second day give the groups two 15-minute feedings of their respective rations. Beginning the third day feed the rations ad libitum for 35 days, at which time the negative control group will be rachitic.

At the end of the feeding period kill the birds with ether. Remove one tibia of each, clean of adhering tissue, number, and place in 95 per cent ethyl alcohol. Crush, wrap individually in filter paper, and extract the bones for 72 hours with hot 95 per cent ethyl alcohol (other solvents may be used for this fat extraction). Dry in a moisture oven, and store in a desiccator. Determine the percentage of ash of the moisture and fat-free bones by igniting in a muffle furnace at 850° for one hour. Compile group ash averages for comparative purposes. (Ash averages for the groups fully protected against rickets should be 30–50 per cent higher than those for the negative control group.)

The following table shows some typical results, such as are obtained in the regular feed inspection activities. All the samples were obtained from retail stocks in Wisconsin, by agents of the Department of Agriculture and Markets.

SUMMARY

The data indicate that the method is of great value in determining relative vitamin D potencies of materials sold as feeding stuffs. Sample No. 3, called dried cod liver oil, is shown to be of no value as a vitamin D carrier. It does not aid mineralization of the tibiae of chickens when fed at a $\frac{1}{2}$ per cent level. Sample No. 1, a cod liver oil, is from two to four times more potent in vitamin D than sample No. 2, sardine oil. At $\frac{1}{8}$ per cent level it produced mineralization equal to $\frac{1}{2}$ per cent addition of

Percentage of ash in tibiae

BASAL RATION	1	2	3	4	5
	BASAL PLUS 1/8%	BASAL PLUS 1/8%	BASAL PLUS 1/8%	BASAL PLUS 1/8%	BASAL PLUS 1/64%
29.6	44.0	32.4	30.1	34.3	30.9
28.7	44.8	35.5	32.7	35.0	32.5
31.5	43.2	35.0	35.0	39.0	33.9
30.3	44.6	34.5	31.8	44.2	31.6
30.6	42.0	36.3	32.4	44.6	33.5
33.0	45.0	36.2	39.4	38.6	32.9
30.0		35.5	31.9	39.8	34.4
29.5		33.8	32.4	33.7	31.0
34.9				40.0	32.6
31.5				41.3	
31.2	Av. 43.9	Av. 34.9	Av. 33.2	Av. 39.1	Av. 32.6
31.8	BASAL PLUS 1/4%	BASAL PLUS 1/4%	BASAL PLUS 1/4%	BASAL PLUS 1/4%	BASAL PLUS 1/32%
33.1					
30.4	41.5	37.6	30.6	45.0	32.2
31.0	45.0	40.2	29.4	42.8	33.2
	44.9	35.8	29.8	38.9	31.6
Av.31.1	45.0	42.6	32.2	42.2	33.4
	47.0	43.4	32.1	45.6	33.8
	46.7	41.8	33.0	42.7	36.1
	44.8	43.7	31.6	45.0	33.0
		37.8	34.6	47.5	31.6
					32.8
	Av. 45.0	Av. 40.4	Av. 31.7	Av. 43.7	Av. 33.1
	BASAL PLUS 1/2%	BASAL PLUS 1/2%	BASAL PLUS 1/2%	BASAL PLUS 1/2%	BASAL PLUS 1/16%
	44.0	44.7	31.2	45.5	36.4
	43.1	44.4	31.2	42.9	38.4
	43.6	45.0	31.6	44.5	34.1
	45.9	46.3	38.2	45.2	33.6
	45.0	45.3	32.6	44.5	42.5
	43.8	46.2	35.5	47.2	35.4
	44.7	43.0	33.8	45.5	36.4
		42.9	26.3	43.5	31.9
					35.1
	Av. 44.3	Av. 44.7	Av. 32.6	Av. 44.9	Av. 36.0
					BASAL PLUS 1/8%
					43.0
					38.2
					43.6
					44.2
					38.0
					38.6
					41.7
					37.3
					42.6
					Av. 40.8

No. 1, Cod liver oil (yellow oil).
No. 2, Sardine oil (from California pilchard).
No. 3, Dried cod liver oil (mislabeled. It is probably a calcium soap in powder form, made from cod liver oil).
No. 4, Cod liver oil (red oil).
No. 5, Cod liver oil claimed to be fortified in Vitamin D potency under a patented process.

sardine oil. Sample No. 4, a cod liver oil, is about one-half as potent in vitamin D as is the cod liver oil represented by sample No. 1. It is more potent than sardine oil, sample No. 2. Sample No. 5, claimed to be cod liver oil fortified in vitamin D potency, is shown to be inferior to sample No. 1, which is regular cod liver oil. At ⅛ per cent addition this sample did not produce as high a mineralization as ⅛ per cent of the regular oil. It is implied that it is eight times more potent in vitamin D than sample No. 4, a regular cod liver oil distributed by the same manufacturer, but it is shown to be little, if any, richer in this vitamin.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method for vitamin D assay incorporated in this report be modified and collaboratively studied.

(2) That any methods which may be suggested for cod liver oil detection in mixed feeds be studied.

REPORT ON HYDROCYANIC ACID IN GLUCOSIDE-BEARING MATERIALS

By G. SMITH (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

In 1920 some collaborative work[2] was done on the determination of hydrocyanic acid from Rangoon beans, which at that time were expected to be imported into the United States in some quantity. The Volhard acid titration method and the Liebig alkaline titration method were tried out by five collaborators, who used the Rangoon beans as a sample material. These beans are a prime example of a product containing cyanogenetic glucosides. The potential hydrocyanic acid content of the beans was estimated at from 440 to 586 parts per million.

As a result of this work the Volhard and Liebig methods were adopted as tentative and the Viehoever and Johns Prussian blue method was likewise adopted.

Since that time no further study has been given to this determination, mostly because it is not one that is made frequently in the course of regulatory work.

In the work described a single sample of linseed meal was analyzed in order to compare the results obtained by the three different methods.

For the digestion and steam distillation of the material, which comprise the first step in all three methods, a plain one liter Florence flask was used instead of a Kjeldahl. The Florence flask is more convenient for steam distillation and there seems to be no reason for specifying a Kjeldahl.

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 44 (1933)
[2] *This Journal*, 4, 151 (1920).

Otherwise the tentative methods were followed closely. The steam generation flask, sample flask, and condenser were set up in a vertical position, connected by the necessary tubing. The method does not describe precisely the set-up of the apparatus, but this way was found to be most convenient. The tube conducting the steam from the generating flask into the sample was terminated in a small bulb with eight small holes pointing sidewise as steam outlets. The set-up for steam distillation can undoubtedly be varied considerably without affecting the results, and the method is correct in not specifying this too closely. During the two-hour maceration process specified by the method the flask should be tightly stoppered. There is evidently some loss of hydrocyanic acid by volatilization during this time, as a strong odor of it is noticeable when the stopper is removed to connect the flask in the steam distillation apparatus. This loss may vary with the temperature, as the solubility of the hydrocyanic acid varies, and it may later be found necessary to specify reasonable temperature limits within which the maceration should be carried out.

Prussian Blue Method

Three duplicate samples of the linseed meal run by the Prussian blue method gave results of from 137 to 148 parts per million of hydrocyanic acid. This method is the longest and most complicated of the three, and therefore offers considerable possibility of error. It is a colorimetric method, and the final material is a suspension of colored material and not a solution.

This suspension has a tendency to settle rapidly and thus make difficult an accurate color comparison. This method, however, is applicable to materials containing volatile chlorides, while the other methods are not, and if carefully handled it may give accurate results.

Alkaline Titration Method

Four samples were run by the alkaline titration method, the results varying from 175.9 to 179.0 parts per million. This method, the shortest and simplest of the three, consists simply of titration of the distillate with silver nitrate solution to the appearance of first turbidity. However, the end point of the titration is a difficult one to fix accurately. Collaborative work will be needed to show how closely results can be checked by this method.

Acid Titration Method

This method consists of receiving the distillate in acidified standard silver nitrate solution and titrating back with potassium sulfocyanate. The end point of this titration, though far from ideal, is not very difficult, and is far more satisfactory than that of the alkaline titration method. The results on four duplicate samples ranged from 155.8 to 157.4 parts

per million, checking more closely than those of either of the other two methods.

EFFECT OF TIME AND ADDITION OF ENZYMES

In order to determine whether a longer time of maceration with water increased the development of hydrocyanic acid, four samples of the meal were macerated for 18 hours (overnight), and the hydrocyanic acid was determined by the acid titration method. To determine the effect of the addition of enzymes, 0.1 gram of emulsin was added to each of four more samples, which were macerated the usual two hours, then distilled and titrated by the Volhard method. Four more samples with the same quantity of emulsin added were macerated 18 hours and the hydrocyanic acid determined likewise.

The results of these determinations show that longer maceration does not materially increase the quantity of hydrocyanic acid evolved, at least in the case of linseed meal, two hours being sufficient. They also indicate that the linseed meal used contained sufficient enzyme within itself to bring about complete decomposition of its glucoside within two hours' time.

SUMMARY

The alkaline titration method is of doubtful reliability, because of the obscure end point.

The Prussian blue method may be accurate within the limits of colorimetry, but it is the least simple and convenient of the three methods. It can be used in some cases where the other methods can not be used.

The Volhard acid titration method appears to be the most accurate and convenient.

Full judgment as to the value of these three methods must await the results of collaborative work.

All analytical results are summarized in the table.

RECOMMENDATIONS[1]

It is recommended—

(1) That the subject of hydrocyanic acid in glucoside-bearing materials be further studied.

(2) That the tentative methods be subjected to collaborative work.

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[1] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 44 (1933).

Analytical Results

(Expressed in parts per million)

Acid Titration Method

| 2 HR. MACERATION | 18 HR. MACERATION | WITH EMULSIN | |
		2 HR. MACERATION	18 HR. MACERATION
156.6	164.5	164.4	157.1
157.4	164.7	164.7	157.9
155.8	164.7	164.7	162.0
156.8	163.9	163.4	160.4
Average 156.7	164.5	164.3	159.4

ALKALINE TITRATION METHOD	PRUSSIAN BLUE METHOD
177.2	
179.0	148.
175.8	145.
176.9	137.
Average 177.2	Average 143.

REPORT ON SOLVENTS FOR DETERMINATION OF FAT IN FEEDING STUFFS

By L. S. WALKER (Agricultural Experiment Station, Burlington, Vermont), *Associate Referee*

A study of the recent literature reveals that very little work has been done in the use of new fat solvents. Dichloromethane and trichloroethylene were suggested. Dichloromethane (methylene chloride, CH_2Cl_2) was chosen for this work as its boiling point is about the same as that of anhydrous ether, being 40° C. against 86.7° C. for trichloroethylene and 35° C. for anhydrous ether.

According to Carlisle and Levine,[1] "Methylene chloride, a low-boiling chlorohydrocarbon, has found extensive use as a general low-boiling solvent. It does not form explosive mixtures with air and on this account may be useful where a safe low-boiling solvent is required and is found stable up to 120° C.; about 80° above its boiling point." Taking these properties into consideration this material lends itself very favorably to the work planned.

Fat determinations were made on 30 different types of feeding stuffs. These were run according to the official method,[2] and both anhydrous

[1] *Ind. Eng. Chem.*, 24, 146 (1932).
[2] *Methods of Analysis*, A.O.A.C., 1930, 279.

ether and dichloromethane were used. Duplicate tests were run, and where there was a difference of more than 0.10 per cent a third test was made. Four results were lower with the new solvent, twenty-five were higher, and thirteen were within 0.10 per cent of each other. The greatest difference was on gluten meal, which favored dichloromethane by 1.48 and 1.83 per cent. The appearance of both extracts was apparently the same, which indicates that more fat was dissolved by the dichloromethane. On the average there was 0.23 per cent more fat dissolved by the new solvent.

The associate referee, therefore, recommends[1] that this work be continued another year.

The following table shows the results.

Anhydrous ether and dichloromethane as a fat solvent in feeding stuffs

	ANHY-DROUS ETHER	DICHLORO-METHANE		ANHY-DROUS ETHER	DICHLORO-METHANE
	pe cen	*per cent*		*per cent*	*per cent*
Cottonseed meal 43%	5.77	6.19	Dried beet pulp	0.52	0.53
Cottonseed meal 41%	6.89	7.13	Alfalfa meal	2.15	2.45
Cottonseed meal 36%	5.18	5.28	Oat mill feed	2.03	1.94
Linseed meal	4.53	4.76	Oat mill feed (sweet)	1.57	1.57
Soybean meal	6.42	6.92	Rolled oats	5.23	5.42
Gluten meal No. 1	2.56	4.04	Rye feed	3.37	3.40
Gluten meal No. 2	2.26	4.19	Corn meal	4.07	4.14
Gluten feed No. 1	4.95	5.28	Dairy feed	4.96	5.04
Gluten feed No. 2	2.76	2.93	Dairy feed (sweet)	4.88	4.84
Brewers' dried grains	7.62	8.21	Stock feed	4.35	4.49
Wheat bran	4.00	4.24	Stock feed (sweet)	3.83	3.75
Wheat middlings	4.98	5.16	Calf meal	4.77	4.87
Wheat mixed feed	4.35	4.35	Meat scraps	10.14	9.66
Wheat red dog	4.48	4.58	Scratch grains	2.41	2.60
Hominy feed	7.20	7.24	Dry mash	5.12	5.25

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 44 (1933).

FIRST DAY

MONDAY—AFTERNOON SESSION

The address of the president, which was delivered Tuesday afternoon, was published in *This Journal*, 16, 27 (1933).

REPORT ON SOILS AND LIMING MATERIALS

By W. H. MacIntire (University of Tennessee Agricultural
Experiment Station, Knoxville, Tenn.), *Referee*

During the past year especial attention has been given the subject of determination of pH values in soils. At the request of the referee, the subject in its relation to the soils of arid and semi-arid regions was handled by P. L. Hibbard, of the University of California. The results obtained by electrometric and colorimetric procedures and a critique and a review of the subject are given in his report. E. F. Snyder of the Bureau of Chemistry and Soils also collaborated by preparing an extensive and useful review of the subject. These two papers constitute contributions that will serve as useful guides and references, and the referee desires to register full appreciation for himself and for this Association. A helpful report on the problem of determining the pH values of acid soils is also submitted by M. F. Morgan, and the referee concurs in his recommendation.

The less-common elements in soils have been studied further by J. S. McHargue, who has perfected a method for the determination of minute quantities of copper. The referee concurs in the recommendation submitted in this report.

It was intended to have a report given on the subject of the wet combustion of organic matter in soils, but illness prevented the completion of the study, which will be continued.

Under the heading of Liming Materials, especial attention has been given to the problem of calcium and magnesium supplements in fertilizers through the use of limestone and dolomite. One paper, "Chemical Changes in Mixtures of Superphosphate with Dolomite and with Limestone," W. H. MacIntire and G. A. Shuey,[1] has appeared, and a second, "Reactivity between Dolomite and Superphosphate Components," W. H. MacIntire and W. M. Shaw, is to appear.[2] At this meeting will be given a paper as a digest of the subject of calcium and magnesium supplements, the factors developed in the two cited publications and the need for methods to determine the amounts of the two supplements incorporated in superphosphate or its mixes.

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[1] *Ind. Eng. Chem.*, 24, 933 (1932).
[2] *Ibid.*, 1401.

In view of the fact that the subject of pH values is being studied intensively by the International Commission of Soil Science, and that none of the present methods has attained perfection, and the further possibility of the new electrodes molybdenum and tungsten,[1] it is recommended[2] that the adoption of a procedure for the determination of the pH value of soils be considered further.

Because of the desirability of a method that will differentiate between total and organic carbon in soils, it is recommended that the subject of wet combustion of soils be studied with the hope that an acceptable method can be recommended for adoption.

REPORT ON REACTION VALUE OF ALKALINE SOILS

DETERMINATION OF HYDROGEN-ION CONCENTRATION OF SOILS WITH THE ANTIMONY ELECTRODE AND A COMPARISON OF THE HYDROGEN AND QUINHYDRONE ELECTRODES ON THE SAME SOILS. ALSO SOME TESTS WITH INDICATORS

By P. L. Hibbard (University of California, Berkeley, Calif.),
Associate Referee

In the beginning it was intended to confine this project to a study of methods for determining the pH of alkaline soils only, but as the work progressed, it entered the field of ordinary soils. It seemed unnecessary and impracticable to keep the study of the two classes separate because the same methods are used throughout.

SOILS USED

Soil 1C, Yolo Silty Clay Loam—Davis, California.

A fine textured, uniform, very productive soil free of so-called alkali or salts. It seems to contain some MnO_2.

Soil 37, Nord Fine Sandy Loam—Chico, California.

Contains about 1% $CaCO_3$ with very little soluble salts, not very productive on account of deficiency of available potassium.

Soils 16, 17, 18, 20, Fresno or Madera Fine Sandy Loams (Differing Chiefly in Content of Soluble Salts and Alkalinity)—Fresno, California.

Soil 16 contains about 2% easily water-soluble sodium salts as NaCl, Na_2SO_4, Na_2CO_3 and $NaNO_3$. It is too alkaline to support plant growth.

Soil 17 is the same as 16 except that it has been leached by flooding in the field so that more than half the sodium salts have been removed, but the alkalinity remains so high that plants do not grow on it.

Soil 18 contains much NaCl and Na_2SO_4 but is only slightly alkaline. Some tolerant plants grow on this soil.

Soil 20+S originally contained a moderate amount of Na_2CO_3 and other salts. It supported some tolerant plants. The sample used in this work was treated with sulfur so that it is now a very acid soil still containing considerable soluble salts.

[1] *Science* (U. S), **76**, No. 1973, p. 356; Trans. Am. Electrochem. Soc., **56**, 201 (1929) and **57**, 361 (1930).
[2] For report of Subcommittee A and action of the Association, see *This Journal*, **16**, 42 (1933).

These six soils were repeatedly tested by the hydrogen and antimony electrodes in the hope of obtaining figures from which to construct a graph for converting the volt readings of the antimony electrode to pH by comparison with the pH found by the hydrogen electrode. The readings with the antimony electrode were too erratic to be used in this way.

A number of other soils were tested by both electrometric and colorimetric methods, but much less work was done with them than with the six above described; in all cases where the methods did not give closely agreeing results, the tests were repeated. These latter soils are of all classes from sands to clays. The results obtained with 17 of them are given in the table.

INSTRUMENTS USED

The potentiometer used for the hydrogen and antimony electrodes was the Leeds and Northrup portable acidity meter No. 7658a with 0.1 N calomel half cell. It may be read in pH direct or in volts. The dial is graduated in millivolts, readable to fifths, and the galvanometer is sensitive to very weak currents so that it is applicable to slightly buffered solutions. The galvanometer sensitivity, as given by Leeds and Northrup, is 7 megohms for the 0.1 N calomel half cell.

For the quinhydrone tests, the Leeds and Northrup pH indicator, Cat. No. 7854, reading millivolts, was used with saturated calomel half cell. The electrode is a gold wire coiled on a glass tube.

The hydrogen electrode used (essentially the Sideris electrode (23)) was a platinum wire about one-half mm. diameter, made into a coil of four turns of about 5 mm. diameter. The wire was sealed into a glass tube about 3 mm. inside diameter, and the upper part of the tube was filled with mercury for connection. This tube was inserted into a larger T tube, 8 mm. in diameter, and made air-tight at the top by means of a rubber tube fitted between the inner and outer glass tubes. The side tube of the outer tube was connected with the hydrogen supply. The bottom of the outer tube was cut off on a slant so that the longer side projected below the platinum electrode, thus protecting it from mechanical injury, while the top of the opening was opposite the platinum electrode so that hydrogen gas bubbling out of the opening caused the electrode to be alternately immersed in the soil suspension and then in the hydrogen gas. The hydrogen, from a cylinder of commercial compressed gas, was purified by passing through a tube filled with platinized asbestos kept at low red heat, then through a water wash bottle, next through an absorption tower of soda lime to remove CO_2, finally to the electrode.

The antimony electrode was a rod about 4 mm. diameter with copper wire soldered to one end. The antimony rod was enclosed in a glass tube from which it projected 2 cm. The glass and antimony were joined by a rubber tube slipped over both. This electrode is described by Barnes and Simon (2).

PREPARATION OF THE SOIL SUSPENSION OF WET SOILS

About 10 grams of the wet soil +10 cc. water was mixed to a cream with mortar and pestle, the mixture was washed into a sample tube to a volume of 40 cc., the whole was well mixed, and 20 cc. was poured into another tube in which the readings with hydrogen electrode were made. Then this portion was returned to the main portion. In this manner the presence of gravel in the portion tested was avoided. For dry soils, 5 gram portions were placed in the sample tube, 20 cc. of water was added, and the mixture was well shaken before the electrode was placed in the suspension. Bailey (1a) recommends that soil be air-dried before pH is obtained.

When tests were made with the antimony electrode, it was dipped in the top one inch of the soil suspension so gravel did not interfere.

For the quinhydrone electrode, the whole mixture was shaken; then a portion of 10–15 cc. was poured off into another tube, quinhydrone was added, and the reading was made as soon as possible, usually within one minute.

For the color determination of pH, the remaining portions of the original mixture were mixed with $BaSO_4$ and filtered. In the clear filtrate, pH was determined by indicators. In this way the same mixture of soil and water was used for determination of pH by all four methods. In some cases, the indicator pH was found by the use of a spot plate method, to be described later.

<div align="center">PROCEDURE</div>

While the readings were being made the mixtures were agitated by hand for the hydrogen and quinhydrone electrodes, and by a mechanical stirrer for the antimony electrode. This stirrer was a glass rod with a slight screw shape at the lower end which dipped in the suspension. The glass rod was rotated on its axis 200 revolutions per minute by a motor so that stirring was vigorous.

In each case after a test had been made, the electrodes and stirrer were washed off with distilled water before being placed in the next soil suspension. The time required to reach electrical equilibrium was longer in proportion as the change in pH between solutions was greater.

For the hydrogen electrode, gas was passed at the rate of 2–5, or more, bubbles per second, and the electrode was standing, with gas passing into the suspension for a few minutes before being connected with the potentiometer, while the previous test was being made. Two electrodes were used; while one was being charged with hydrogen, the other was being read on the potentiometer. In this way, there was little waiting for the hydrogen electrode, which usually came to equilibrium in a minute or two.

Much shorter time is required for the hydrogen electrode to reach equilibrium in going toward more alkaline readings than when it is going from alkaline toward more acid readings.

The antimony electrode usually came to approximate equilibrium as soon as the setting could be made, that is, in one-half minute or less with solutions of similar pH, but when there was an unusual change in pH from one solution to the next, several minutes were sometimes required. The quinhydrone electrode usually reached approximate equilibrium within a few seconds, but the time was much longer when the change in pH from one mixture to the next was great. After apparent equilibrium with any of the methods had been reached, there was sometimes fluctuation of a few millivolts, or a drift in one direction.

In some cases, as with soils 1C and 38, the quinhydrone electrode gave readings much too high (probably caused by MnO_2). This was checked repeatedly. Even the hydrogen electrode may give fairly steady readings which are far from correct.

<div align="center">EFFECT OF RATIO OF SOIL TO WATER ON pH</div>

In soils having very low buffer power, increase of water in the mixture may change the pH considerably unless the water has very nearly the

same pH as the soil. For this reason, neutral water free of CO_2 should be used. Most soils are sufficiently buffered so that a little CO_2 in the water has little effect on pH of the soil in $\frac{1}{2}$ suspension. In clay or fine textured soils, a ratio of 1:4 is preferable as making the mixture more fluid, thus easier to handle. Sandy soils may be very well managed with 1 part of soil to 2 parts of water.

PECULIARITIES IN BEHAVIOR OF THE ANTIMONY ELECTRODE

The antimony electrode is sensitive to temperature changes. In a buffer of about 11 pH, it gave the following readings: t. 22, .683 v.; t. 32,. 698 v.; t. 28, .691 v.; t. 25, .685 v. In acid solutions, temperature changes make less difference. Stirring the solution greatly aids in reaching equilibrium quickly.

With some soils there is a wide range between the first reading and the final equilibrium reading as in Soil 18, .480 to .510 v. In this case, there is apparent almost equilibrium at first, then a gradual drift toward the final equilibrium, which is slowly reached. When the difference in the pH from one solution to the pH of the next solution is small, equilibrium is almost instantaneous, and is more likely to be correct than when the change is great. There is likely to be considerable variation (5–20 mv. = .05 − .20 pH) in readings on the same solution at different times. In the work here reported, two similar electrodes made as previously described were used. Later a somewhat different electrode was used.* All of them gave about the same readings and seemed to work equally well.

With the same electrode but with a saturated KCl calomel cell and Weston voltmeter divided to .01 v. and readable to .002 v., same stirrer as before, the results seemed somewhat better in most cases. Equilibrium was reached rather quickly, except for Soil 1C, when a little drifting was apparent. Soils which contain considerable salts come to equilibrium more rapidly than do those containing little salts. In going from a material of one pH to a very different one, the electrode changes potential slowly at first from that of the previous to that of the new solution, but there seems to be no difference, whether from the acid or the alkaline side.

With standard Clark and Lubs buffers, pH 3–8, the antimony electrode shows close agreement in most cases with the quinhydrone electrode in the same solutions. With soils, the agreement is good below pH 7, but above 7 the antimony electrode is somewhat uncertain, relative to the hydrogen electrode and with the quinhydrone electrode in the range where quinhydrone is applicable.

Generally the antimony electrode readings in the range pH 3–7 are correct to ± 0.1 pH. Above pH 7, it may be -0.1–0.8 pH off. No means of knowing where it is correct was found. In most cases in the higher

* Supplied by Leeds and Northrup through the kindness of Mr. N. Cohn.

alkalinities, the antimony electrode is nearly right. Leeds and Northrup state, in a private communication, that there is likely to be considerable difference in the indications of the antimony electrode between buffered and unbuffered solutions of about the same *p*H.

In order to convert the E.M.F. of the antimony electrode to *p*H, a graph was constructed from the volt readings on a number of buffer solutions plotted against the *p*H of the same buffers as obtained by the hydrogen electrode on repeated tests with both the antimony and the hydrogen electrodes. This seemed preferable to the method most investigators have used, i.e., making the conversion by means of an equation. There are about as many different equations as there have been investigators.

INDICATOR METHOD

As there is likely to be some difference in *p*H between the solid and the liquid in a suspension of soil in water, a test on the filtered extract is not usually considered very accurate. Two methods may be used, the spot plate and clarification by $BaSO_4$, Kuehn's (19) method. The indicator method is able to give only approximate results with soils that have a colored extract, such as black alkali soils.

Spot Plate Method

Place 0.5 gram of soil in each of three depressions in a spot plate. Add to each 1 cc. of water and mix with a small glass rod. Add 1 drop of the three indicators most likely (one above, one correct, and one below) to cover the *p*H range of the soil. By a slight jarring motion, cause the drop of indicator to mix with the liquid over the soil without being mixed with the soil itself. After a few minutes' standing, the supernatant liquid will usually be clear enough to permit a fairly good comparison with the color of the same indicator added to an appropriate buffer in a neighboring depression on the spot plate. In this manner it is possible to obtain within a few minutes a sufficiently accurate measure of the *p*H of a soil for almost any practical purpose. If buffers are lacking, comparison may be made with a color chart such as is supplied by Clark (8). Still better are the colored glass Hellige standards or the standard indicator buffer tubes supplied by La Motte and others. Another scheme is the Wulff test made in Germany. With this test a celluloid strip impregnated with the indicator is pressed into the moist soil for a moment, then washed off, and the color is compared with a color chart.

Kuehn Method (19), Using $BaSO_4$

To a ¼ suspension of the soil in water, add $BaSO_4$, as used for X-ray work (nearly colloidal), and shake the mixture. Usually more $BaSO_4$ than soil is needed in order to effect clarification quickly. As soon as the solid matter settles, add the indicator to the partly clear top liquid, or pipet a portion of the liquid into a small tube, add the indicator, and compare the mixture with any of the devices above mentioned in connection with the spot plate method. A blank test on the $BaSO_4$ should be made to be sure that it is neutral and free of soluble salts which might affect the *p*H:

In soils over *p*H 8 or in clayey soils, this method of clarification is not very successful. It works much better with sandy and acid soils.

The spot plate method is simpler, quicker, and probably about as accurate as the BaSO₄ method, and may be preferred because it adds nothing which might change the *p*H of the soil.

To obtain exact results with slightly buffered soils, isohydric indicators (1) should be used, though for practical purposes this is an unnecessary refinement.

EXAMINATION OF INDICATORS FOR THE RANGE *p*H 8.5–12

For the range 8.9–9.5, thymol blue is perhaps best, though phenolphthalein does fairly well. On soils it seems to give lower readings than does thymol blue, as compared with readings on buffer solutions. Cresol phthalein covers nearly the same range, perhaps not so well. Alkali blue 9.4–12 shows a good color change from strong blue at 9 to pale blue at 11, yet seems not to work well with soils. Alizarine (Schultz 778, National Aniline and Chemical Company) gives fairly good color changes, yellow to purple, in the range 9.5–12. Alizarine Yellow R covers about the same range, but is not so good. It is yellowish to brownish red, 9.5–10.5; the colors are too pale. Azo blue, 10.5–11.5, and alpha naphthol benzine, 8.5–9.8, do not seem to be useful for soils.

In the range 9–12, none of these indicators gives such sharp color changes as do those used for *p*H 3–8, such as bromcresol green, or bromthymol blue. Differences of less than 0.5 *p*H unit are not easily detected in the range 9.5 to 12. The dark color derived from some black alkali soils seriously interferes with the use of any indicator for determining *p*H.

RESULTS OF *p*H MEASUREMENTS

In the following table are given some of the results obtained by the methods described. Nearly all the tests were repeated, some of them several times, and the figures given are those which were thought to be most reliable. There is much discordance, but the reason is not evident. In some cases, such as soils 1C, 38 and 101, the Feigl (12) test for MnO₂ with benzidine indicated the quinhydrone readings might be too high on account of MnO₂. When this test did not show MnO₂, the quinhydrone results agreed much better with the hydrogen electrode. Several workers have shown that MnO₂ in the soil vitiates the quinhydrone results. The Feigl test and the H₂O₂ test may be used to distinguish such soils.

The best printed description of electrometric methods is that of the International Society of Soil Science (10). Following the description of methods are tables of results obtained by the Committee of the International Society of Soil Science, *Soil Research*, 2, 84–139 (1930). Agreement among the various workers' results is not so good as might be desired. Tests of 100 soils by various methods are given by Utscher (26).

pH of plant nutrition soils found by four methods

SOIL NO.	ELECTROMETRIC			INDICATOR
	QUINHYDRONE	HYDROGEN	ANTIMONY	
20+S		3.70	3.82	
1C	7.4	6.74	6.58	6.7
18	7.8	7.43	7.43	7.6
37	8.21	8.20	8.05	7.8
17	—	9.83	9.50	10+
16	—	9.99	10.10	10+
21	6.42	—	6.50	6.8
29	7.94	—	7.90	7.8
30	6.94	—	6.90	6.7
35	5.36	5.38	—	5.6
36	6.76	6.7	6.50	6.5
38	7.19	6.65	6.6	6.7
40	6.26	—	6.23	6.5
53	7.11	—	7.1	7.0
59	5.97	5.72	—	6.1
64	6.94	6.60	6.6	7.0
65	7.23	7.15	7.20	7.0
78	4.91	4.8	5.15	5.0
80	8.30	—	8.1	8.0
90	5.79	—	5.8	6.2
95	7.86	7.78	7.55	7.7
101	6.84	6.70	6.6	6.7
102	6.03	6.7	6.2	6.6

RECOMMENDATIONS AND SUGGESTIONS

The hydrogen electrode and the quinhydrone electrode systems are now common and fairly well understood apparatus for measuring pH of soils. Yet there is great variety in the electrodes themselves, in the methods of operating them, in the methods of preparing the soil suspension to be measured, as well as in other important details. Within the present limits of knowledge and experience, it is not yet possible to select the best. Also, the best for one set of conditions may not be the best for some other set of conditions. Therefore, it appears that the most that can be done about it at present is to suggest some definite procedures for trial with the hope that time and experience will enable the analyst to make a selection of the best.

The antimony electrode offers great promise. If it can be made reliable without too great cost of time and money, it may supersede both the other methods for soils, as well as for many other purposes. It has the

advantages of simplicity, cheapness, and ruggedness. There is no need to add anything to the solution which will vitiate it for some other use and no need for hydrogen. Rapidity of attaining equilibrium, freedom from injury by poisoning, and general adaptability to all sorts of solutions are other advantages. Although several workers report excellent results with it, many find that it is not always reliable. Until the details necessary to make it dependable are known, it seems best not to include it as one of the instruments for official sanction, although it may be used for approximate results.

For most practical purposes indicators may be relied upon to measure the *p*H of soils with sufficient accuracy; precision is not easily attainable with them, but usually it is not necessary. Owing to the dark color of the water extract of many black alkali soils, it may sometimes be difficult or even impossible to measure their *p*H by means of indicators. Besides, good indicators are not available for high alkaline range. However, this is unimportant because plants cannot live in very alkaline soil. At present it seems unwise to describe precisely the method of using indicators to determine soil *p*H. The glass electrode was not studied. It may, if freed from some of its limitations, expense, fragility, and complexity of apparatus, etc., become very desirable for determining the *p*H of soils.

With these limitations in mind, the associate referee offers the following methods for the approval of the Association.

METHODS

Part I

The methods submitted by the associate referee under Part I are those formulated by the Committee of the International Society of Soil Science. As they have been published in *Soil Research*, *2*, pp. 81–83, and 142–143 (1930), it is not necessary to publish them in *This Journal*.

Part II

METHODS SUGGESTED BY P. L. HIBBARD

The Antimony Electrode Method

The apparatus is the same as that used for the hydrogen electrode except that hydrogen is omitted, and a rod of metallic antimony is used instead of the platinum electrode. As results obtained by this device are of uncertain validity it seems unwise to give precise directions at present.

Parks and Beard (20) have made a critical study of this method and find it good under certain conditions. The vacuum tube potentiometer aided greatly in obtaining constant results. From these writers and from other sources has been gathered the following information in regard to use of the antimony electrode:

1. The solution should be in equilibrium with ordinary air. Variation of air in the solution causes variation of potential.

2. Presence of even minute quantities of mercury or its salts in the solution is likely to poison the electrode and lead to erroneous results.

3. Most workers report more constant results when the solution is in motion than when still. Stirring should always be at the same rate.

4. Temperature influences the antimony electrode considerably and more as the solution is more alkaline, therefore it should be kept constant.

5. It seems fairly well established that the antimony electrode gives somewhat divergent readings in buffered and unbuffered solutions of the same pH. For this reason a graph prepared by the use of buffer solutions for converting volt readings to pH may give erroneous values when the electrode is applied to unbuffered materials such as some very poor soils.

6. In many soils there is some difference in pH between the top portions of a water suspension and the mud in the bottom of the container. Much more concordant results are likely to be had by using the top suspension only, instead of lowering the electrode into the thick mud at the bottom. Mix 1 part soil and 2 of water for a minute or two. Let stand about a quarter of a minute, pour off into the electrode vessel the upper portion of the suspension, leaving all the coarser portion of the soil. The decanted portion is used for pH determination. It should be well mixed by shaking immediately before placing the electrode in it, and the suspension should be stirred all the time the readings are being made.

The details of the spot plate and Kuehn's method will not be repeated here, as they are essentially as given previously in this report.

REVIEW OF LITERATURE

No attempt has been made by the writer to review all the available literature of hydrogen-ion measurements. Some of the articles pertinent to the antimony and the quinhydrone electrodes are mentioned.

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REPORT ON REACTION VALUE OF ACID SOILS

THE PRESENT STATUS OF SOIL ACIDITY METHODS

By M. F. MORGAN (Connecticut Agricultural Experiment Station, New Haven, Conn.), *Associate Referee*

This paper presents a general picture of the present status and main characteristics of soil acidity methods which are being used in soil investigational work. No attempt to present the details of technic used in the individual methods will be made. Hence, this may be regarded as an introduction to more specific comparative studies by this organization.

The soil acidity problem is now being attacked along the following fronts:

1. The intensity of soil acidity, in terms of hydrogen-ion concentration or *p*H.

2. The amount of lime required to bring an acid soil to a desired reaction, normally the neutral point.

3. The quantitative factor in soil acidity, in terms of exchangeable or replaceable hydrogen and its relationship to the base exchange capacity of the soil.

4. Indirect factors in soil acidity phenomena, such as the solubilities of aluminum, iron, and manganese.

HYDROGEN-ION CONCENTRATION METHODS

During the past fifteen years determinations of the intensity factor in soil acidity on the basis of hydrogen-ion concentration (pH) have come to be the most widely used of any single measurement of a soil property. The relationship between the pH value of a soil and numerous factors pertaining to its fertility has probably justified the attention given to this phase of soil investigation, and the simplicity of a number of the methods has permitted its development.

However, as much confusion has arisen through the multiplicity of methods now being used, a few comments upon this situation may be pertinent.

The earlier work of an exact character was largely done by electrometric methods, gas hydrogen electrodes developed from the original Hildebrand (5) type being used. These were slow, required careful technic, and were subject to errors due to electrode poisoning and disturbances of soil equilibria through the expulsion of carbon dioxide by the hydrogen gas. Nevertheless, much fundamental research was produced by this type of method.

The application of the quinhydrone electrode to pH measurements of soils, introduced by Biilmann (1), provided a simple, rapid, and generally satisfactory technic for soils which are not more alkaline than 8.0 pH. This method has come into quite general use in practically all states with important areas of acid soils, and it appears to give reliable results, in harmony with those obtained by the gas hydrogen electrode, with soils which do not contain unusual amounts of manganese dioxide. Such soils are readily recognized in the quinhydrone electrode procedure, through the rapid drift of potential which is encountered.

In order to provide reliable results on such soils, the antimony and glass electrode methods have been adapted to soil investigations. The former is extremely simple, but it is of questionable accuracy when very exact results are demanded. The glass electrode requires a very sensitive electrometer, batteries, and rheostat units in addition to the usual equipment used in other electrometric methods. The actual measurement is extremely simple and rapid, and is subject to none of the errors involved in other methods. It possesses the additional advantage of providing a hydrogen-ion concentration measurement without introducing any contaminating material into the sample. This appears to be a promising method for use in checking results obtained by the quinhydrone electrode.

Colorimetric methods are in general use where an accuracy of from 0.1 to 0.2 pH is satisfactory. The use of barium sulfate in obtaining a clear soil extract of hydrogen-ion concentration comparable to that obtained

by electrometric methods gives promise of greater reliability for the colorimetric method. It has the advantage of simplicity and inexpensiveness of apparatus. The colorimetric procedure is also well adapted to field tests of only approximate accuracy.

LIME REQUIREMENT METHODS

The obvious practical requirement in soil acidity studies is the correct evaluation of the amount of lime required to neutralize a given amount of soil. This is usually expressed in terms of pounds or tons of lime or limestone required to neutralize 2,000,000 pounds of dry soil (the arbitrary weight per acre to plow depth).

Numerous lime requirement methods have been devised. Agreement with one another and the actual lime requirements obtained in field trials have been frequently disappointing. The present concept of the nature of soil acidity demonstrates that the results of different methods are dependent upon the type of soil acidity which they actually measure. For instance, the titratable acidity released from a soil by treatment with potassium nitrate, as in the Hopkins method (7), is dependent upon exchange acidity, while treatment with calcium acetate, as in the Jones method (9), also measures hydrolytic acidity, which in many cases is responsible for the major portion of the soil's ability to decompose carbonates.

The Jones method (9) is probably more widely used than any other, and gives results which provide a satisfactory basis for field recommendation, except on occasional abnormal soils. Various workers have introduced several modifications of the technic originally described at the first meeting of this Association. These should be carefully considered if the Jones method is to be given an official sanction.

The Veitch method (14) was formerly in much favor, since it measured the absorptive capacity of the soil for lime water. The tedious nature of its technic and the artificiality of the temperature conditions as compared with the field have discouraged its retention as a routine method for lime requirement determination.

The Hutcheson and MacLennon method (8) should be mentioned, since it is used rather extensively abroad. On theoretical grounds it has much to commend it, since it measures the liberation of carbon dioxide from calcium bicarbonate at normal temperatures. Results obtained are usually somewhat lower than those obtained from the Jones method.

Numerous other methods have fallen by the wayside, or have persisted in only one or two institutions, which have retained them for personal preferences or special situations. Among these are those of Hopkins, Daikuhara (4), Manns (10), Tacke (12), Bouyoucos (2) and Truog (13).

BASE-UNSATURATION METHODS

The development of present knowledge of the base exchange properties of soil has provided a fundamental theoretical basis for the quantitative

measurement of soil acidity in terms of exchangeable hydrogen and relative base-unsaturation. For instance, in the case of the Jones method, the titratable acidity of an aliquot of the liquid obtained in the treatment of a soil sample with a dilute solution of calcium acetate must be multiplied by an arbitrary factor. On the other hand, the total titratable acidity developed in successive leachings of calcium acetate through the soil requires no factor since it represents the total replacement by calcium of the hydrogen in the base exchange complex. It is now obvious that soil acidity must be expressed in relative terms, with respect to the total base exchange capacity of the soil. Thus two soils with the same content of exchangeable hydrogen, may be totally different in their relative base-unsaturation. Many research workers now believe that the degree of harmful acidity in soils is largely a function of the percentage of the total base-exchange capacity which may be accounted for as replaceable hydrogen.

Much investigation is now being conducted along these lines. Methods employed are almost as numerous as the individuals who are attacking the problem. Most of the methods for exchangeable or replaceable hydrogen involve leaching the soil with neutral salts. The barium acetate method proposed by Parker has met with much favor, and is fairly well adapted to routine work.

Exchangeable hydrogen may be also determined indirectly, by difference between the total exchangeable bases and the total base exchange capacity, as proposed by Hissink (6). Total base exchange capacity methods usually involve the saturation of the soil with a single basic ion such as ammonium or barium, and its subsequent determination. Total exchangeable bases may be replaced by leaching the soil with ammonium salts or dilute acids. They may be also removed by electrodialysis.

INDIRECT METHODS

Soils which are acid to any considerable degree are characterized by a marked increase in ferric and aluminic ions which are readily soluble, either in water, alcohol, or dilute acids of low dissociation. Since at least part of the harmful properties of strongly acid soils are believed to be due to the toxic effects of the increased solubility of aluminum, a direct measurement of the status of the soil in this respect is an essential approach to the complete delineation of the soil acidity problem.

The Comber thiocyanate test (3), of approximately quantitative character, indicates soil acidity in terms of its effects upon the solubility of the ferric ion. The writer (11) has recently proposed a roughly quantitative measure of the solubility of the aluminic ion. Exact quantitative methods for soluble aluminum are long and exacting in technic. The use of the "aluminon" reagent has simplified them somewhat, by enabling a colorimetric procedure to be followed.

The solubility of manganese is another important factor in acid soils. The quantitative measurement of manganese involves no special problems, but the solution used for extracting manganese from the soil in solubility studies is a matter of question. The writer has recently worked out a simple microchemical technic, using 0.5 N acetic acid buffered at 4.6 pH with sodium acetate. This gives promise of diagnostic value on extremely acid soils.

The above brief picture of the situation is sufficient to show the manifold nature of the soil acidity problem and the consequent requirements for adequate, sound, and reproducible methods for the determination of pH, lime requirement, exchangeable hydrogen, total base exchange capacity, and aluminum solubility. In the main these requirements are being met. There is a great lack of uniformity in technic along all of these lines. However, each year has brought new developments, which would be impossible had standardized procedures been adopted which might have been based on fundamentally unsound principles. When the present state of flux in regard to soil acidity is considered, it seems that there is no position of certainty with respect to the proper evaluation of the methods.

Certain procedures, such as the quinhydrone electrode method for pH and the Jones lime requirement method, are widely used and becoming fairly well standardized in technic. Within their inherent limitations, a tentative acceptance of such methods by this organization might be feasible within the next year or so.[1]

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No report on liming materials was given by the associate referee. See report of the Referee on Soils and Liming Materials.

REPORT ON LESS COMMON ELEMENTS IN SOILS

By J. S. McHARGUE (Kentucky Agricultural Experiment Station, Lexington, Ky.), *Associate Referee*

During the past year considerable time has been devoted to the determination of iodine in soil by the combustion method. The analytical procedure was carried out by D. W. Young, Assistant Chemist in the Department of Chemistry of the Kentucky Agricultural Experiment Station, working under the direction of the associate referee. A paper describing the combustion method for the determination of iodine in soil, together with comparative results obtained by the Andrew[1] and the fusion methods, respectively, has been published.[2]

Fig. 1 shows the apparatus for the combustion method, ready for use: 1 is an electric combustion-tube furnace having a 50-mm. hole through the center; 2, a quartz combustion tube, inside diameter 40 mm., outside diameter 50 mm.; 3, a sillimanite combustion boat, 250 by 32 by 20 mm.; 4, a rheostat; and 5, gas wash bottles containing a 5 per cent solution of potassium carbonate. Three bottles are used. The last wash bottle is attached to a suction pump which draws air through the apparatus during the combustion. The rheostat controls the rate of heating the furnace. About one hour is required to bring the furnace to the maximum temperature of 1100° C.

From 25 to 100 grams of soil (or a similar quantity of limestone or marl) is weighed into the combustion boat. The amount of sample to use depends on whether the iodine content is high or low. The sample is heated for about 1 hour after the furnace reaches the maximum temperature. Maintenance of the full heat is essential. The wash bottles are then disconnected and the solution is rinsed into a porcelain dish and evaporated to dryness. The residue is dissolved in a few milliliters of hot

[1] *Analyst*, **55**, (647) 269 (1930).
[2] *Ind. Eng. Chem. Anal. Ed.*, **4**, 214 (1932).

water and filtered into a small porcelain dish, evaporated to dryness again, and ignited gently to destroy the small amount of organic matter usually present. When cool, the residue is dissolved in a few drops of hot water and filtered into a small separatory funnel, and pure 95 per cent ethanol is added until a layer stands on top of the aqueous solution. After being shaken vigorously for about 2 minutes, the two layers of solution are allowed to separate, the aqueous layer is run into a small empty separatory funnel, and the extraction repeated twice. The residue from evaporating the combined alcoholic solutions to dryness is dissolved in a few drops of water, transferred to a small separatory funnel, and acidified with sulfuric acid. A few drops of sulfurous acid are added, and the funnel is stoppered and shaken. Then exactly 1 ml. of carbon disulfide and approximately 1 ml. each of sulfuric acid (1+1) and 10 per cent sodium nitrite solution are added, and the funnel is shaken vigorously for about 1 minute. If the carbon disulfide is colored pink a portion is compared in
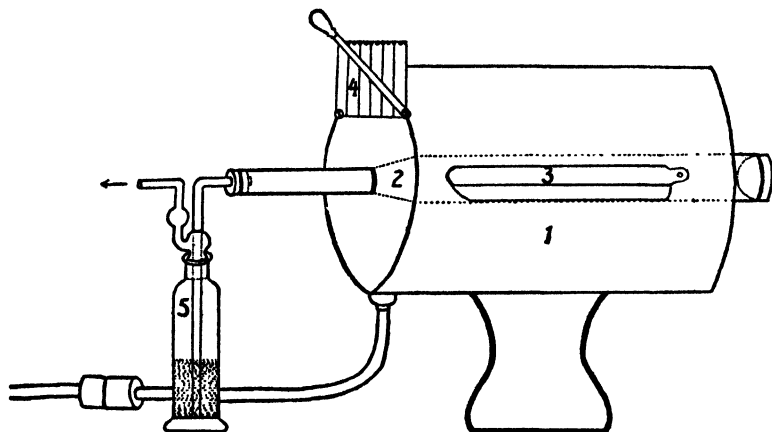

FIG. 1.—FURNACE READY FOR USE.

a microcolorimeter with an iodine standard treated in the same way. The iodine content is calculated to parts per million.

To ascertain the accuracy of the combustion method in comparison with the method of Andrew and the fusion method, a considerable number of duplicate determinations were made on several different soils by each of these methods. The results are contained in Table 1.

The Andrew method consists in heating gently in a nickel dish 1 gram of soil to which 1 ml. of a saturated·solution of potassium carbonate has been added, until all the water is expelled and the organic matter has been destroyed. After being cooled the residue is digested in hot water, the solution is boiled and filtered, and the insoluble residue washed twice. The filtrate is evaporated to dryness and ignited gently, and the residue is cooled and extracted with 90 per cent ethanol. The alcoholic extract is

evaporated to dryness, the residue is taken up in a few drops of water and transferred to a separatory funnel, iodine is liberated in the usual way and absorbed in chloroform, and the color is compared with an iodine standard treated in a like manner.

The fusion method consists in adding 15 grams of potassium hydroxide and a little water to 5 grams of finely pulverized soil in an iron crucible and heating cautiously until the water has been expelled and the silicates are completely decomposed. The fused mass is slaked with enough water to form a sludge of the alkaline silicates. A small quantity of a saturated solution of sulfurous acid is added, and the silicate sludge is made acid by the addition of hydrochloric acid to decompose the alkaline silicates. The silicate sludge is then made distinctly alkaline by the addition of a saturated solution of potassium carbonate. The mass of silica, aluminum, and iron hydroxides is transferred onto a folded filter and thoroughly washed with hot water. The filtrate is evaporated to the point where soluble salts begin to crystallize out. The solution is then transferred and rinsed into a separatory funnel. Pure 95 per cent ethanol is added until two distinct layers of solution are formed. The mixture is vigorously shaken, the aqueous portion is separated, and two extractions are made. The combined alcoholic extracts are evaporated to dryness in a small porcelain dish, heated gently until charred, and cooled; the residue is dissolved in a few ml. of hot water and filtered into a 30 ml. separatory funnel. The solution is acidified with sulfuric acid and 2 ml. of a saturated solution of sulfurous acid is added. The funnel is stoppered and vigorously shaken to reduce iodates to iodides. Then exactly 1 ml. of carbon disulfide and approximately 1 ml. each of sulfuric acid (1+1) and 10 per cent sodium nitrite solution are added, and the funnel is shaken vigorously for about 1 minute. If the carbon disulfide is colored pink, a portion is compared in a microcolorimeter with an iodine standard treated in the same way. The iodine content is calculated to parts per million.

<div align="center">

TABLE 1

Results of determinations of iodine in 12 samples of soil by three methods

(Parts per million of air-dried soil)

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SOIL SAMPLES		ANDREW'S METHOD	FUSION METHOD	COMBUSTION METHOD
1		3.80	4.15	3.73
		4.00	4.12	3.80
	Average	3.90	4.13	3.76
2		5.00	5.70	5.64
		4.82	5.64	5.80
		4.73	5.58	5.40
	Average	4.85	5.64	5.61

3		3.50	4.20	4.00
		3.50	4.23	4.00
		3.34	4.01	4.12
	Average	3.45	4.15	4.04
4		3.84	5.50	5.60
		3.63	6.10	5.40
		3.20	6.00	5.40
	Average	3.56	5.87	5.47
5		3.60	3.50	3.32
		4.76	3.55	2.95
	Average	4.18	3.52	3.14
6		7.30	8.10	8.00
		7.20	8.12	8.15
	Average	7.25	8.11	8.08
7		6.45	8.55	8.50
		6.36	8.56	8.00
	Average	6.41	8.56	8.25
8		4.50	5.60	5.60
		4.50	5.66	5.46
		4.76	5.75	5.30
	Average	4.59	5.67	5.45
9		4.56	5.25	5.30
		4.70	5.36	5.05
		4.75	5.56	5.40
	Average	4.67	5.39	5.25
10		7.80	7.00	6.77
		7.00	7.20	6.82
		6.95	7.16	7.00
	Average	7.25	7.12	6.86
11		2.00	2.30	2.70
		2.10	2.70	2.79
		2.14	2.80	2.90
	Average	2.08	2.60	2.79
12		4.10	5.06	4.82
		4.31	4.96	4.90
		4.02	5.19	4.70
	Average	4.14	5.07	4.81
Average of 32 deter- minations		4.60	5.41	5.23

DISCUSSION OF RESULTS

The results show that in most instances the fusion method gave slightly higher figures for iodine content than did the combustion method, but the differences are not material. Only 3 of the 12 samples gave a higher result by the Andrew method than by the combustion method, and in only two were slightly higher figures obtained by the Andrew method than by the fusion method.

From the procedure described in the Andrew method, it would not be expected that total iodine would be obtained because all the silicates would not be decomposed by the low heat applied in the fusion procedure. Andrew states that in no case was he able to recover more than 60–70 per cent of the total iodine present, and he attributes this to losses from overheating. It is possible, however, that the low results obtained by the Andrew method are due, at least in part, to incomplete decomposition of the soil silicates. The writers are of the opinion that as much as 95 per cent recovery of the total iodine content of soils is possible by either the fusion or the combustion method.

The principal advantage of the combustion method is that the tedious manipulation of extracting a small quantity of iodine from a relatively large mass of silicate material is obviated. It requires about 1 hour to make a combustion after the furnace has attained the maximum temperature. By having several combustion boats, it is possible to run samples continuously by removing the ignited sample and inserting a fresh portion without allowing the furnace to cool. In this way it is possible to make six or more combustions per day with a single-tube furnace. Since electric furnaces carrying as many as four tubes are on the market, it is possible to increase this number of determinations per day accordingly.

To ascertain whether iodine was retained by the soil after ignition in the electric furnace, 5 gram portions of ignited soil were finely ground and fused in an iron crucible with potassium hydroxide as previously described, but no iodine was found. These tests show that iodine can be completely volatilized at 1100° C. from residual soils such as occur in Kentucky.

The amount of current used in making a determination is about 6 kw-hr. This makes the cost for current in this laboratory about 20 cents a determination.

Six principal geological areas occur in Kentucky. Soils from each of the areas were analyzed by the combustion method for iodine. Table 2 shows a summary of the iodine content of soil from the six areas.

Fifty-eight of the above samples of soil were analyzed for iodine by the fusion method and gave an average of 4.70 p.p.m. of iodine. The same number of determinations on the same samples by the combustion method gave an average of 4.44 p.p.m. for iodine. Out of 409 independent dupli-

TABLE 2
Summary

AREAS	NO. OF SAMPLES ANALYZED	IODINE (PARTS PER MILLION)		
		MAXIMUM	MINIMUM	AVERAGE
1. Purchase	42	6.93	1.59	4.57
2. W. Coal Field	69	7.37	2.31	4.11
3. Mississippian	100	16.95	2.53	6.10
4. O. Bluegrass	132	11.85	1.10	4.07
5. I. Bluegrass	64	8.25	2.40	4.35
6. E. Coal Field	14	3.08	0.80	2.05
Total	421	16.95	0.81	4.59

cates, 323, or 80 per cent, did not differ more than 0.3 p.p.m.; and 262 duplicate determinations, or 64 per cent, differed less than 0.2 p.p.m.

The associate referee recommends[1] that the combustion method for the determination of iodine in soil be made tentative and be subjected to cooperative study next year and that the fusion method be made an alternative method under the same conditions.

REPORT ON FERTILIZERS

By G. S. Fraps (Agricultural Experiment Station,
College Station, Texas), *Referee*

The Referee on Fertilizers has acted only in an advisory capacity. The associate referees will present their work and recommendations. Although they have been laboring under some difficulties this year they have made progress and are to be commended.

As this organization has been working on the methods of analyses for fertilizers for over forty years, it would seem that progress could no longer be made. The methods are indeed among the most accurate possessed by the Association. However decided changes have been made in some of them in the last few years and there is opportunity for further work. The methods for distinguishing between the different forms of nitrogen have been elaborated and improved; the methods for the activity of nitrogen in fertilizers have been made more detailed; and the citrate method for available phosphoric acid has been reviewed and changed in order to allow the use of larger quantities of liquid ammonia in fertilizers without at the same time lowering the standard of availability. These are some of the changes which have been made in recent years.

Work proposed by the associate referees includes further improvement of the methods for distinguishing the different forms of nitrogen and of the methods for analyzing highly concentrated fertilizers. Preparation of

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[1] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 43 (1933).

ammonium citrate requires further attention, and the estimation of free phosphoric acid in superphosphate appears to be a desirable problem. The estimation of filler in mixed fertilizer has never been taken up, and it is an open question whether it should be. Some states require a statement of the percentage of filler. Some agronomists recommend limestone as a filler, although experience has shown that the use of large quantities of limestone may revert the phosphoric acid and cause the available phosphoric acid to run far below guarantee. Such, at least, has been the experience of the referee.

Problems still remain to be solved concerning the analysis of fertilizer, and other problems will arise with the progress of science and the march of industry. The method of collaborative research has given results in perfecting methods of which this organization may well be proud.

REPORT ON PHOSPHORIC ACID
PREPARATION OF NEUTRAL AMMONIUM CITRATE SOLUTION

By Wm. H. Ross, *Associate Referee*, and K. C. Beeson (Fertilizer and Fixed Nitrogen Investigations, Bureau of Chemistry and Soils, Washington, D. C.)

At the last meeting of this Association a recommendation was adopted[1] that a collaborative study be made of the relative accuracy and convenience of the two procedures given in the last edition of *Methods of Analysis* for the preparation of neutral ammonium citrate solution.

The use of neutral ammonium citrate solution as a means of measuring the fertilizer value of phosphatic fertilizers was first proposed by Fresenius, Neubauer and Luck[2] in 1871.

The method used in preparing this solution, as described in the Proceedings of the first meeting of this Association,[3] consisted in neutralizing citric acid with ammonium carbonate, boiling to expel carbon dioxide, and adjusting to the neutral point by the addition of ammonia or citric acid as required, using litmus paper as indicator. At the third meeting of the Association a report was presented by Richardson[4] on tests made with litmus and six other indicators in the preparation of neutral citrate solution. Corallin was considered to be superior to any of the indicators used in the tests, and it was recommended that the use of this indicator be made official. It was also recommended that the specific gravity of the solution be determined at 20° C. These recommendations were adopted, and the corallin method appears as the first of the alternative methods for preparing neutral ammonium citrate solution in all three editions of *Methods of Analysis, A.O.A.C.*

[1] *This Journal*, **15**, 44, 227 (1932).
[2] *Z. anal. Chem.*, **10**, 149 (1871).
[3] Proc. Convention Agr. Chem., Sept 8–9 (1884).
[4] U. S. Dept. Agr. Div. Chem. Bull., **12**, 21 (1886).

Four years later Huston[1] recommended an alternative method which involves the use of an alcoholic solution of calcium chloride and cochineal. This method was promptly adopted, and it is listed as the second of the alternative methods for preparing neutral ammonium citrate solution in the first two editions of *Methods of Analysis, A.O.A.C.* It was also recommended at the same time that the use of ammonium carbonate in the preparation of citrate solution be replaced by ammonium hydroxide.

In 1920 the Referee on Phosphoric Acid presented a report[2] on a collaborative study of these two methods and of several others that subsequently appeared in the literature. The methods studied in addition to the official methods were Hand's[3] azolitmin method; Patten and Marti's[4] titration method; and Eastman and Hildebrand's[5] rosolic acid method with color standard. Tests were also made with a solution prepared by diluting a solution of crystallized triammonium citrate to a sp. gr. of 1.09 at 20° C.

The solutions prepared by these different methods were found to vary widely in reaction. Those that were most acid gave relatively low results for citrate-insoluble P_2O_5, while those that were alkaline gave values that were relatively high. It was accordingly recommended[6] that the work be continued with a view to determining the exact composition and method of preparing a strictly neutral solution of ammonium citrate.

The study that was undertaken in accordance with this recommendation is described by Robinson,[7] Associate Referee, in three excellent reports that were presented at the three succeeding meetings of this Association. These reports show that the normal salt of ammonia and citric acid is alkaline in reaction due to hydrolysis; that the ratio of ammonia to anhydrous citric acid in a strictly neutral solution of ammonium citrate is 1:3.794; and that such a solution can be most conveniently prepared by use of buffered standards with phenol red.

The method recommended by Robinson was adopted[8] by the Association in 1924 and appeared in the 1925 edition of *Methods of Analysis, A.O.A.C.*, as the third of the optional methods given for preparing neutral ammonium citrate solutions. In 1927 action was taken to delete the calcium chloride method,[9] and the method of using buffered standards with phenol red therefore appears in the last edition of *Methods of Analysis, A.O.A.C.*, as the second of the two alternative methods for preparing neutral citrate solutions.

The importance of insuring a neutral reaction in ammonium citrate solutions was again emphasized in the report that was submitted last year

[1] U. S. Dept. Agr. Div. Chem. Bull., **31**, 101 (1891).
[2] *This Journal*, **3**, 279 (1920).
[3] U. S. Dept. Agr. Bur. Chem. Bull., **132**, 11 (1910).
[4] *J. Ind. Eng. Chem.*, **5**, 567 (1913).
[5] *Ibid.*, **6**, 577 (1914).
[6] *This Journal*, **4**, 562 (1921).
[7] *Ibid.*, **5**, 92 (1921); 433 (1922); **6**, 384 (1923).
[8] *Ibid.*, **7**, 265 (1924).
[9] *Ibid.*, **10**, 64 (1927); **11**, 68 (1928).

on the availability of ammoniated superphosphates. It was shown that the solubilities of the di- and tricalcium phosphates occurring in ammoniated superphosphates vary considerably with the pH of the citrate solution, and the view was expressed that solutions prepared by the corallin method are likely to vary too greatly in reaction for the accurate evaluation of the phosphatic materials now on the market. This view is amply supported by the work that has already been done by this Association and a recommendation for the deletion of the method would seem to be warranted without further collaborative study. In order, however, that a direct comparison might be made of the two methods, each of the analysts who collaborated on last year's work was asked to submit a neutral ammonium citrate solution prepared by each method.

The wording of the first or corallin method, as submitted to the collaborators, was the same as that given in *Methods of Analysis, A.O.A.C.* The wording of the second method was changed slightly to conform more closely with what is now considered good practice. Thus the modified directions, which are published[1] elsewhere, specify that the pH determination shall be made on the solution itself and not on the solution diluted with three parts of water as directed in the original wording of the method.

The values for the pH of the solutions submitted by the collaborators as determined by the electrometric and colorimetric methods are given in Table 1.

The results given in Table 1 show (1) that the solutions prepared by the first or corallin method vary greatly in reaction; (2) that all solutions prepared by the second method, when phenol red is used, do not vary more than 0.1 unit from a pH of 7.0; (3) that the mean of the values found by this method agrees within 0.1 pH unit with the corresponding mean found by the electrometric method; and (4) that a minus correction of 0.2 pH unit must be made to compensate for salt error when bromthymol blue is used as indicator in the second method.

Although the second of the official methods gives results that are slightly higher than those obtained with the electrometric method, it is felt that this method is sufficiently accurate for routine work in the evaluation of phosphates.

The first of the official methods was reported to be unsatisfactory by all the collaborators listed in Table 1 and by a number of others who collaborated in last year's work.

CITRATE-INSOLUBLE PHOSPHORIC ACID

At the last annual meeting a recommendation was adopted that a further study be made of the method for citrate-insoluble phosphoric acid

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[1] *This Journal*, 16, 68 (1933).

with a view to modification that will secure more concordant results. One of the principal sources of error in this method arises from the difficulty sometimes experienced in filtering off the citrate-insoluble residue. Considerable attention has been given to this problem during the past year, but no satisfactory method has yet been found for filtering the residues obtained in the analysis of materials that contain colloidal phosphatic components such as steamed bone meal, tricalcium phosphate, and heavily ammoniated superphosphates.

TABLE 1

Reaction of ammonium citrate solutions

COLLABORATOR	ELECTROMETRIC (H+ ELECTRODE) pH		COLORIMETRIC pH			
			PHENOL RED		BROMTHYMOL BLUE	
	NO. 1	NO. 2	NO. 1	NO. 2	NO. 1	NO. 2
J. G. Asher, Virginia-Carolina Chemical Corp.		7.01		7.1		7.3
C. A. Butt, International Agricultural Corp.	6.68	6.83		6.9		7.1
R. D. Caldwell, Armour Fertilizer Works	6.49	7.07		7.1	6.8	
W. C. Geagley, Michigan State Dept. of Agr.	5.75	7.03		7.1		7.3
R. S. Gifford, American Cyanamid Co.		6.83		6.9		7.2
C. C. Howes, Davison Chemical Co.	6.41	6.85		6.9	6.7	7.2
R. E. Ingham, F. S. Royster Guano Co.	6.67	6.87	6.8	7.0	7.0	7.2
W. Catesby Jones, Virginia State Dept. of Agr.	6.73	6.89	6.8	7.0	7.0	7.3
H. R. Kraybill, Indiana Agr. Expt. Station	6.86	6.87	6.9	7.0	7.2	7.2
R. S. Lamb, Darling and Co.	5.18	6.81		6.9		7.1
C. Neutzel, F. S. Royster Guano Co.	6.61				6.9	
L. F. Rader, Bureau of Chemistry & Soils	7.93		8.0	7.0		7.2
Mean		6.91		7.0		7.2

Ford and Kraybill[1] have recently shown in a comparative study of the different types of filter papers now on the market that while some are suitable for use in the analysis of the usual commercial fertilizers, none of the 15 types examined proved satisfactory for filtering the citrate-insoluble residues from tricalcium phosphate and other phosphatic materials of a colloidal nature.

[1] *This Journal*, 15, 653 (1932).

RECOMMENDATIONS[1]

It is recommended—

(1) That the first of the alternative methods given in *Methods of Analysis, A.O.A.C., 1930*, p. 11, sec. **13**, (1) be deleted (first action).

(2) That the wording of the second of the alternative methods given in *Methods of Analysis, A.O.A.C., 1930*, p. 11, sec. 13 (2), be changed as suggested and as published previously in *This Journal*, **16**, 68 (1933).

(3) That the study of the method for citrate-insoluble phosphoric acid be continued.

(4) That a collaborative study be made of methods for determining free acid in superphosphates.

REPORT ON NITROGEN

By A. L. PRINCE (Agricultural Experiment Station, New Brunswick, N. J.), *Associate Referee*

In last year's report the following recommendation was left open for further consideration: "That a further attempt be made to devise a method suitable for control work to determine accurately the ammoniacal nitrogen in the presence of urea and cyanamid." Although the associate referee has given considerable thought to this problem during the present year, no new developments or positive results have been forthcoming. Last year's work indicated that even when the amount of magnesium oxide used in the distillation of ammonia was materially reduced, the decomposition of the urea still persisted. Later on it was suggested by Shaw and MacIntire[2] that the presence of calcium compounds as an impurity in the ordinary C. P. magnesium oxide might account for this condition. However, it was found by the associate referee that a solution of urea will decompose to a slight extent with liberation of ammonia on boiling even when no base at all is added. Consequently, no further attempt in this direction was made.

Another possible mode of attack is the separation of urea in the mixed fertilizer by means of alcohol. This is the process outlined by Moore and White.[3] In their procedure the residue from the alcohol extract is leached with water and the ammoniacal nitrogen determined on the water extract in the usual manner. Now, although ammonium sulfate is nearly insoluble in alcohol, other ammonium salts, especially ammonium nitrate, are slightly soluble. Under such conditions two distillations for ammonia nitrogen would be necessary, one from the water extract and the other from the alcohol extract. Even assuming that the results so obtained are nearer the correct ammonia nitrogen figure, the associate referee doubts the practicability of such a procedure for ordinary control work.

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 46 (1933).
[2] *Ind. Eng. Chem.*, **20**, 315 (1928).
[3] *Ibid.*, **19**, 264 (1927).

The time factor, as well as the inconvenience of arranging the stills for the alcohol distillation, are the main objections.

It appears that the present method of determining ammonia nitrogen, as incorporated in the Robertson procedure, is the best that can be offered until a new modification can be suggested.

Another problem in connection with nitrogen determinations came to the attention of the associate referee early this year. It concerned the use of selenium as a catalyst in the determination of total organic nitrogen by the Kjeldahl method. The original article was by M. F. Lauro.[1] The particular advantage claimed for this catalyst is that the time for digestion is materially lessened. Often the digestion can be completed in 15–30 minutes or less, and there is no need for the use of potassium sulfide in the subsequent distillation as is the case when mercury is used as the catalyst. Since only a small amount of selenium is needed (0.1–0.2 gram), its use would hardly be more expensive than mercury, possibly less. The associate referee made a few preliminary analyses on some miscellaneous samples of plant material with the following results:

Comparison of mercury and selenium as catalysts in the total nitrogen determination

MATERIAL ANALYZED	MERCURY AS CATALYST (0.65 GRAM)	SELENIUM AS CATALYST (0.2 GRAM)
	per cent	*per cent*
Wheat straw	0.379	0.381
Wheat grain	1.934	1.939
Buckwheat	1.259	1.251
Cabbage	2.251	2.259
Florida peat (1 hour digested)	2.694	2.766
Florida peat (1½ hours digested)	2.704	2.734

The results obtained with the two catalysts appear to be quite concordant, and the selenium seemed to be much more efficient than the mercury in the digestion process. In most cases, 30 minutes or less was necessary for the digestion. In connection with this method correspondence was carried on with R. A. Osborn of the Bureau of Chemistry and Soils, Washington, D. C. In a recent letter Osborn made the following comments: "We have carried out a series of experiments with flour samples in which the selenium catalyst has been compared with mercuric oxide.[2] In general, our results compare quite closely with those of R. M. Sandstedt.[3] We see little advantage of the selenium catalyst over the mercuric oxide from the standpoint of time required for digestion or accuracy of results." Referring to the abstract of Sandstedt's article, the following comments are of interest: "A series of determinations was made on a high protein flour and on a sample of ground bran with 25 cc. of

[1] *Ind. Eng. Chem. Anal. Ed.*, 3, 401 (1931).
[2] *This Journal*, 16, 110 (1933).
[3] *Cereal Chem.*, 9, 156 (1932); *C. A.*, 26, 3044 (1932).

H_2SO_4, 12–13 grams of K_2SO_4, and the catalyst (0.1 gram Cu, 0.7 gram HgO, or 0.1 gram Se). The digestion time was varied from 30 to 150 minutes, the most intense heat obtainable from the gas being used (natural gas). The digestion of the samples with selenium and with mercuric oxide was almost complete in 30 minutes and was complete in 45 minutes, while the digestion with copper was complete in one hour. It appears that there is greater danger of losing nitrogen by extremely long digestion with selenium than with other catalysts. Selenium has the advantage over mercuric oxide of not requiring a precipitant in distillation."

The associate referee believes that it would be desirable to try out this catalyst on fertilizer materials and mixed fertilizers in comparison with mercury. The time necessary for digestion with various materials as well as the amount of selenium to use should be studied.

RECOMMENDATIONS[1]

It is recommended—

(1) That the associate referee continue to study any new processes which may lead to a more accurate determination of ammoniacal nitrogen in the presence of urea and cyanamid.

(2) That a comparative study be made of the catalysts selenium and mercury in the determination of total nitrogen in fertilizer materials and mixed fertilizers.

Since preparing this report a recent article on the subject "Selenium in Determination of Nitrogen by Kjeldahl Method" by Tennant, Harrell, and Stull has appeared in the literature.[2] The authors compared the use of selenium and copper sulfate in the Kjeldahl method on the extracts of various kinds of plant pollen, potato flour, green pepper, horse and guinea pig epithelium, and urine. The following quotation concerning the results is significant:

Analyses of extracts, on which parallel nitrogen determinations were run using copper sulfate and amorphous precipitated selenium, showed checking results with a decrease of one-half to two-thirds the time necessary for digestion where selenium was used. Owing to the variation in burners and the fact that heating in some cases was carried on longer than necessary in order to assure complete digestion, the time factor is not absolute. The results were considered sufficiently conclusive to adopt the modification in these analyses.

From these results it would appear that selenium was equally as efficient as copper sulfate, but since there was variation in the amount of heat applied during the digestions, it would hardly be fair to claim a superiority for the selenium.

REPORT ON HIGH ANALYSIS FERTILIZERS[1]

By JOHN B. SMITH (Agricultural Experiment Station, Kingston, R. I.), *Associate Referee*

The work of the past year has been a repetition of work reported in 1931.[2] The tentative Bidwell-Sterling procedure[3] was studied further, samples of fertilizer materials sold under the trade names of calcium nitrate, calurea, and urea being used. The samples had been stored in sacks for several months under humid conditions to simulate conditions existing at times in the trade, and the nitrate of lime had absorbed considerable moisture.

Samples weighing from 10 to 20 grams were taken from covered weighing tubes and placed in the distillation apparatus devised by Bidwell and Sterling,[4] except that glass joints in place of cork were provided for the apparatus. The moisture was distilled with toluene during periods varying from 10 to 20 hours.[2] The urea and calurea were ground rapidly to pass a 10-mesh sieve, and the nitrate of lime was used both in this state and unground.

Urea and calurea contained less than 1 per cent of moisture, and this was obtained by distillation for 5 hours. The only difficulty noted was a tendency for urea to sublime and collect on the condenser tube. Slow distillation at the start helped to prevent this. Results for these materials were in accord, although the degree of accuracy was not great. Fertilizer analysts will be interested only in the effect of such low-moisture materials on high-moisture ingredients in mixture, and no such mixtures were studied. Calurea is not such a mixture, as it is made by crystallizing a solution containing calcium nitrate, urea, and a small proportion of ammonium nitrate together. The resulting crystals contain very little water of hydration.

The results for nitrate of lime were less satisfactory than those reported previously. Moisture in different portions of the unground material varied from 18 to 22 per cent. The variation was less after the material had been ground to pass a 10-mesh sieve, but satisfactory agreement was not obtained. The difficulty was found to be caused by the variation in moisture between small and large particles in the sample. After separation by sifting rapidly through a 10-mesh sieve, it was found that the small particles contained materially more moisture than the larger. All attempts at grinding, however, resulted in a noticeable absorption of moisture. Until some means can be devised for reducing the size of the sample particles without exposure to humid air, the method cannot be made exact. No collaborative work was attempted for this reason.

[1] Contribution No. 433 of the Rhode Island Agricultural Experiment Station.
[2] *This Journal*, 15, 272 (1932).
[3] *Ibid.*, 45, 66.
[4] *Ibid.*, 8, 295 (1924).

UNIFORMITY OF HIGH ANALYSIS FERTILIZER MIXTURES

The work of the previous year was repeated. A single core was taken from each of 25 sacks in a large shipment of an 8-16-14 fertilizer sold by a reputable manufacturer. Each sack was placed on its side, the closed sampler was thrust diagonally through the contents in a horizontal plane, and approximately 120 grams was removed. The samples were tightly stoppered in glass, and later ground to pass a 1-mm. sieve. Analyses were made by the methods of this association.[1]

The results, listed in Table 1, are the averages of duplicate determinations, no two having been made upon a sample simultaneously. The prob-

TABLE 1

Analysis of individual cores taken from each of 25 sacks of an 8–16–14 fertilizer*

SERIAL NUMBER OF BORING	NITROGEN	TOTAL PHOSPHORIC ACID (P_2O_5)	WATER-SOLUBLE POTASH (K_2O)
	per cent	per cent	per cent
1	7.63	16.55	13.27
2	7.88	16.94	13.37
3	7.71	16.94	13.75
4	8.19	17.76	12.54
5	7.75	16.26	14.82
6	7.67	16.84	13.28
7	7.80	16.41	13.81
8	7.64	16.12	13.04
9	8.00	16.41	13.88
10	7.62	16.17	13.45
11	7.88	16.75	14.10
12	7.61	16.46	13.25
13	7.56	16.60	14.07
14	7.89	17.23	13.67
15	8.19	17.86	13.03
16	7.63	17.09	13.55
17	7.99	17.28	14.40
18	7.70	16.36	14.98
19	8.04	17.96	13.40
20	7.64	16.65	13.42
21	7.63	16.65	13.53
22	7.94	16.65	14.39
23	7.79	16.70	13.60
24	8.09	17.18	13.86
25	7.89	17.23	13.59
Mean	7.81 ±0.03	16.84 ±0.07	13.68 ±0.07

* Analyses by W. L. Adams and A. S. Knowles, Jr.; calculations by D. R. Willard.

able errors include analytical errors as well as sampling errors. Average differences between the 25 pairs of duplicate determinations were 0.03, 0.07 and 0.07 for nitrogen, total phosphoric acid (P_2O_5), and water-soluble

[1] *Methods of Analysis, A.O.A.C.*, 1930.

potash (K_2O), respectively. By chance these are exactly similar in magnitude to the probable errors for the means of the respective analyses, showing that errors of analysis were a material part of the total errors.

As percentages of the means the probable errors are 0.32, 0.40, and 0.54 per cent for N, P_2O_5, and K_2O, respectively. Corresponding percentages for the 1931 series of samples were 0.12, 0.43, and 0.95. The brand sampled in 1932 was the more uniform of the two with respect to phosphoric acid and potash, but not for nitrogen.

The degree of accuracy required for fertilizer analyses is a matter of opinion, and uniform standards have not been established. From the data reported in 1931 it was calculated that analysis of a composite sample of 14 cores from 25 sacks would assure probable errors of approximately ±0.1 per cent of P_2O_5 and K_2O in the 8-16-16 grade of fertilizer studied, and a smaller probable error for nitrogen. It happens that the same is true for K_2O in the 8-16-14 grade sampled in the current year, and that 12 borings would be sufficient for total P_2O_5. The probable error for nitrogen in such composites would be much less. If sampling accuracy is proportional to the square root of the total number of units in a lot[1] and 14 are required from 25 units, approximately three times the square root of the number of sacks in the shipments studied should be sampled to give probable errors of less than ±0.1 per cent for total phosphoric acid (P_2O_5) and water-soluble potash (K_2O). One third of this number would be sufficient for nitrogen.

The number of units apparently required for the degree of accuracy chosen above is larger than that usually sampled in fertilizer practice, and more evidence is required before definite conclusions may be reached. Assuming that it proves representative of general conditions it is possible that some improved method of sample preparation can be devised. Finer grinding of the 1931 samples gave small increases in the percentages of potash found, but did not improve the uniformity of the 25 individual analyses. Accuracy materially greater than probable errors of ±0.1 per cent for two of the fertilizer constituents could not have been attained in these instances without refinement in analytical methods, for these remain the same regardless of the number of cores in the composite. It is apparent, however, that the sampling error was greater than the analytical errors.

RECOMMENDATIONS[2]

It is recommended—

(1) That the determination of moisture in hygroscopic materials, especially mixtures of calcium and ammonium nitrates, be studied further.

(2) That the uniformity of the parcels comprising fertilizer shipments be studied collaboratively.

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[1] *This Journal*, 11, 220 (1928).
[2] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 47 (1933).

(3) That the consensus of opinion regarding the degree of accuracy practicable for the analysis of shipments of high-analysis fertilizers be determined.

REPORT ON POTASH

By L. D. HAIGH (Agricultural Experiment Station, Columbia, Mo.), *Associate Referee*

This paper will serve merely as a progress report. No samples were sent out to collaborators as it was thought best to study first the effect of some of the modifications suggested by others.

The common criticism directed against the present official procedure for potash in mixed fertilizers, known as the Lindo-Gladding method, is that all the water-soluble potash actually added in the preparation of a mixture is not found in the finished mixture. The extraction of the water-soluble potash from the fertilizer mixture has been discussed in recent sessions of this Association, and various suggestions have been made.

Formerly the method of preparation of the solution called for placing the sample in the volumetric flask, adding water, heating the mixture to the boiling point, and maintaining it thus for 30 minutes. This long contact of the water-soluble potash with other materials of the fertilizer mixture at the boiling point was suspected of being a contributory cause of low results for potash, and it was therefore proposed by Breckenridge[1] in 1909 that the sample be washed on a filter with successive additions of boiling water until a volume of 200 cc. of filtrate is obtained. This proposal was adopted, and it is the present official procedure for preparation of the potash solution. Some chemists now claim that this procedure does not remove the potash as effectively as it should. They state that if the washings be continued until about an additional 100 cc. has been passed through the filter, higher results for potash can be obtained. A trial in this laboratory of these two procedures on a few samples seems to substantiate these statements. The following results were obtained.

SAMPLE	WASHINGS TO 220 CC. *per cent* K_2O	WASHINGS TO 300 CC. *per cent* K_2O
No. 1, 4–12–4 mixture	4.78	4.87
No. 2, 4–16–4 mixture	4.03	4.07
No. 3, 15–30–15 mixture	16.24	16.29

These results are averages of four determinations, with one exception, that of an average of three determinations.

An attempt was made to increase the effectiveness of the washings by washing the sample by decantation preliminary to washing on the filter. Some trials of this method are promising as in some cases slightly higher

[1] *J Ind Eng. Chem*, 1, 409, 804 (1909).

results were found than by the official method of washing. The results are much the same as obtained when the washings are continued to an additional volume of 100 cc. over the official procedure.

Another possible source of low results for potash has been pointed out by Bible.[1] The water washings carrying the water-soluble potash will also contain ammonium phosphate. When the residue from the evaporated aliquot is ignited, ammonia is evolved, and the residual phosphoric acid is changed to metaphosphoric acid on ignition. As the phosphoric acids are less volatile than sulfuric acid, metaphosphate of potash is produced, and as this is soluble with difficulty it may be filtered out before the addition of the potassium chloroplatinate, thus causing low results. Bible proposes to avoid this formation of metaphosphate by the addition to the aliquot drawn for the ignition of sufficient sodium hydroxide to combine with all the phosphoric acid present. This difficulty was of course expected to develop most frequently when the ratio of phosphoric acid to potash was high. For the purpose of studying the effect of this procedure, samples of a 2-16-2 and of a 4-16-4 fertilizer were analyzed, the official procedure being used. The addition of sodium hydroxide to the aliquot, as suggested by Bible, was also tried. The results obtained were as follows:

SAMPLE (Platinum dishes used for ignition)	REGULAR OFFICIAL METHOD *per cent*	OFFICIAL METHOD, NaOH ADDED *per cent*
2-16-2	2.23	2.22
4-16-4	4.41	4.21

Thus no additional value for potash was obtained on these two samples by the use of sodium hydroxide before ignition.

Lockhart[2] claims that the disturbing effects of ignition on the results for potash can be avoided by preventing the temperature of ignition from rising too high. Experience seems to point out that ignition is not always a cause of variations, but if the quantities of potassium and sodium oxide are low in comparison with ammonium salts it is evident that it is a real factor.

Articles discussing the official method for potash have attributed low results obtained to occlusion of potash by the heavy precipitate which forms on the addition of ammonium hydroxide and ammonium oxalate. Tests of this precipitate by chemists of this Association have failed to reveal any significant amount of potash occluded by it. In this laboratory the average of sixteen tests for potash in the precipitate gave a result corresponding to 0.002 per cent potassium oxide based on the weight of the sample, while the highest result found corresponded to 0.01 per cent potassium oxide. It is believed that significant sources of error in the official method are due to other causes than occlusion of potassium oxide by the precipitates.

[1] *J. Ind. Eng. Chem. Anal. Ed.*, 4, 234 (1932).
[2] *Ibid.*, 3, 407 (1931).

Some years ago this Association gave attention to the effect of varying strengths of alcohol used to wash the chloroplatinate precipitate of potash. R. N. Brackett[1] reviewed this work in an article to which the reader is referred. The question as to whether the major variations in the potash results by the official method are not due after all to the effects of the alcohol washings rather than to the other operations of the method is again being raised by fertilizer chemists. One chemist suggests that the alcohol used for washing be saturated with potassium chloroplatinate before use. This idea was first proposed by Davis[2] in his work on the perchlorate method for potash. The possibility of variations in the potash results, due to different strengths of alcohol used, makes it necessary to follow strictly the directions for alcohol washing as now used if the effect of different modifications of preparation of solution or igniting the residues in the preliminary steps of the method is to be observed correctly.

Modification of the official method for potash is frequently suggested to the Referee on Fertilizers. Whether these suggestions are worthy of collaborative study must be determined by some preliminary trials by the chemists of the Association interested in the method.

Some years ago the Sherrill centrifuge method was proposed for study as an official method.[3] The associate referee is impressed with the value of this procedure as a control method in factory operations, but as the potash result is obtained indirectly it does not seem suitable for official work.

The method for the estimation of chlorine in fertilizers was adopted as official (first reading) at the meeting of the association in 1928. No further collaborative work has been conducted since that date, but this method has been in general use by official and control chemists since its introduction and has given entire satisfaction.

RECOMMENDATIONS[4]

It is recommended—

(1) That further time be given to try out suggested modifications of the official method in order that some of these may be used in collaborative study.

(2) That the method for the determination of chlorine in fertilizers (official method, first reading) be adopted as official (final action).

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[1] *This Journal*, **7**, 382 (1924).
[2] *Ibid.*, **1**, 406 (1915).
[3] *Ibid.*, **6**, 376, 402 (1922).
[4] For report of Subcommittee A and action of the Association, see *This Journal*, **16** 47 (1933).

CONTRIBUTED PAPERS

DETERMINATION OF ARSENIC IN PLANT MATERIALS

By R. B. DEEMER and J. A. SCHRICKER (Bureau of Chemistry and Soils, Division of Soil Fertility, Washington, D. C.)

INTRODUCTION

Experience of previous workers with the Gutzeit method for the determination of arsenic in foods has demonstrated the need for a more satisfactory procedure for the accurate determination of arsenic in micro quantities. The difficulty with the Gutzeit method lies in duplicating the brown arsenic stains on the mercuric bromide strips. It is only by using very carefully controlled uniform conditions that even approximate results can be obtained. Robinson[1] combined the arsine evolution of the Gutzeit method with the estimation of the arsenic, so separated, by Denige's[2] colorimetric method. Denige's method was improved by Truog and Meyer[3] and later by Zinzadze.[4]

In seeking a method to be used in the analysis of plant material the writers thought that a combination of these methods offered possibilities. It is also considered that recent[5] attention to crop responses to arsenic applications and the prominence attained by the rare or less common elements warrant the presentation of the details of this investigation.

Attempts to separate the arsenic as arsine from solutions obtained by the official method[6] of acid digestion of plant materials were not successful. However, the details of the arsine evolution method used in this work are given with the hope that others interested may continue the study of the subject. On the other hand, an application of the well-known arsenic trichloride distillation to a solution obtained by the acid digestion of plant material gave an excellent separation of arsenic as estimated by Zinzadze's colorimetric method.

METHODS

DIGESTION OF SAMPLE

Fifty grams of the dry material was wet thoroughly with water in an 800 cc. Kjeldahl flask and digested according to the official procedure with these modifications: An initial volume of 100 cc. of nitric acid and 10 cc. of sulfuric acid was used and more acid added in small portions as needed. A total of 300 cc. of nitric acid and 25 cc. of sulfuric acid was necessary to complete oxidation. Instead of adding the ammonium oxalate in solution, 2 grams of the salt was added to the flask after the cooled contents had been diluted with 100 cc. of water.

[1] W. O. Robinson, personal communication.
[2] Compt. rend., 171, 802, (1920); Compt. rend. soc. biol., 84, 875 (1921).
[3] Ind. Eng. Chem. Anal. Ed., 1, 136 (1929).
[4] Ann. Agron. 1st yr., No. 3, N.S., 321 (1931).
[5] W. R. Paden, J. Soc. Agron., 24, 363 (1932).
[6] Methods of Analysis, A.O.A.C., 1930, 109, 307.

When finally brought to fumes and cooled the contents were washed into a 250 cc. volumetric flask, cooled, and made up to volume. This was Solution (a) used in the arsine evolution method.

In the arsenic trichloride distillation method the procedure was continued from the point where the digestion was finally brought to fumes after the addition of the ammonium oxalate.

ARSINE EVOLUTION METHOD

APPARATUS

The apparatus consisted of a 200 cc. Erlenmeyer flask joined to a Winkler spiral by means of a ground-glass stopper having a perforated curved drip tube.

REAGENTS

(a) *Stannous chloride solution.*—40 grams of $SnCl_2$ in 100 cc. of HCl.

(b) *Potassium iodide solution.*—15 grams of KI in 100 cc. of water.

(c) *Copper sulfate solution.*—10 grams of $CuSO_4 \cdot 5H_2O$ in 100 cc. of water.

(d) *Nitric acid.*—Concentrated, saturated with bromine.

(e) *Zinc.*—As-free, 20-mesh.

(f) *Sulfuric acid.*—1 per cent by volume (approximately 0.36 *N*).

(g) *Sodium carbonate.*—Aqueous solution, approximately 0.36 *N*.

(h) *Beta-dinitrophenol.*—Saturated aqueous solution.

(i) *Monopotassium arsenate*, (KH_2AsO_4). Analyzed, standard solution containing 0.01 mg. of As_2O_5 per cc.

(j) *Molybdenum blue.*[1]—6.02 grams of molybdenum trioxide was dissolved in 120 cc. of sulfuric acid (sp. gr. 1.785) in a 300 cc. Kjeldahl flask by gentle boiling and occasionally rotating the flask The solution was cooled, poured into 70 cc. of water, cooled again, and made up to 200 cc. in a volumetric flask (Solution I). Another 200 cc. of Solution I was prepared and returned to the 300 cc. Kjeldahl flask and boiled gently for 10 minutes with 0.56 grams of pure powdered metallic molybdenum. The solution was then cooled and decanted from the undissolved metal into a 200 cc. volumetric flask and made to volume (Solution II). The molybdenum blue reagent (Solution III) was obtained by mixing quantities of Solutions I and II so that 2.55 cc. of the resulting mixture just decolorized 0.2 cc. of normal potassium permanganate. This was done by running a test titration with Solution II on 0.2 cc. of the normal permanganate and then calculating the quantity of Solution I to be added to a given quantity of Solution II. The resulting mixture (Solution III) was checked with another titration. The prepared reagent was then filtered through a sintered glass filter with the aid of suction.

PROCEDURE

A 100 cc. aliquot of Solution (a) from the acid digestion of plant material was pipetted into the evolution flask, and 0.5 cc. of the stannous chloride solution and 1 cc. of the potassium iodide solution were added. The mixture was brought nearly to a boil and kept hot on the steam bath for 30 minutes. One cc. of the copper sulfate solution was added, then 8 grams of zinc, preferably through a funnel to prevent granules of zinc getting into the ground-glass joint. The flask was connected with the Winkler spiral containing 10 cc. of the nitric acid-bromine solution. A few drops of water placed on the ground-glass joint assured tightness and prevented sticking when the spiral was disconnected. After the first violence of the reaction was over (about 15 minutes), the apparatus was transferred to the steam bath, which was maintained at a gentle heat, and evolution was continued for about 2 hours or until

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[1] Modification of Zinzadze's procedure, *loc. cit.*

complete. The spiral was then disconnected and washed into a 150 cc. beaker with water. By connecting the spiral to a raised wash bottle with a siphon efficient washing was completed with less than 100 cc. of water. The solution and washings were evaporated on a hot plate at low heat to about 2 cc. and completed to dryness on the steam bath. The residue was taken up, first with 1 cc. of concentrated nitric acid, and evaporated until no odor of nitric acid persisted, then with 1 cc. of 1 per cent sulfuric acid and evaporated on the steam bath until the residue no longer flowed when the beaker was tilted. It was washed with warm water into a 50 cc. volumetric flask and 3 drops of beta-dinitrophenol were added; then it was neutralized with sodium carbonate solution to a very faint yellow and 0.6 cc. of molybdenum blue reagent was added. Finally it was made to approximately 40 cc. and heated on the steam bath for 30 minutes. The flask was cooled, made to 50 cc., and the depth of color was read in a colorimeter, there being used as standard an aliquot of a solution of monopotassium arsenate evaporated down, treated with nitric acid and 1 per cent sulfuric acid, and color developed as with the sample. Best results were obtained with amounts between 0.1 and 0.2 mg. of arsenic pentoxide, as the color between these limits was most easily read, a solution depth of 50 mm. for 0.2 mg. and 80 mm. for 0.1 mg. being used. The solution from the spiral was made up to a given volume, and an aliquot was taken for subsequent treatment if the arsenic pentoxide content exceeded 0.2 mg.

(For test evolutions upon standard solutions a sulfuric acid concentration of 5 per cent by volume is optimum.)

ARSENIC TRICHLORIDE DISTILLATION METHOD

APPARATUS

The apparatus consisted of the Kjeldahl flask used in the digestion, and a Hopkin connecting bulb with a 3 mm. delivery tube extending to the bottom of a 100 cc. cylinder as receiver. The end of the delivery tube had a 1 mm. opening.

REAGENTS

(a) *Sodium chloride.*—30 grams.

(b) *Cuprous chloride.*—2 grams.

(c) *Potassium bromide.*—0.5 gram.

(d) *Hydrochloric acid.*—Concentrated, 25 cc.

(e) *Nitric acid.*—Concentrated, saturated with bromine (for treatment of the distillate).

PROCEDURE

This method followed closely the distillation procedure of Taber[1] except that 2 grams of cuprous chloride[2] was substituted for ferrous sulfate. After addition of 20 cc. of water and the reagents the arsenic trichloride was distilled from the digestion flask through the Hopkin connecting bulb into the 100 cc. cylinder containing 80 cc. of water. When the volume of solution in the cylinder reached 95 cc. the distillation was complete, the length of time required being 10 to 15 minutes. The distillate was evaporated in a 150 cc. beaker on a hot plate with nitric acid-bromine solution added in small quantities at intervals. Final dryness was attained on the steam bath, followed by treatment with 1 cc. of nitric acid-bromine solution and a second evaporation to dryness. The procedure was continued, as in the arsine evolution method, with the treatment of the residue with nitric and sulfuric acids and subsequent color development.

[1] *This Journal,* 14, 436 (1931).
[2] W. W. Scott, Standard Methods of Chemical Analysis. 2nd ed., 33 (1917).

RESULTS

The analytical results found on pure solutions of monopotassium arsenate and on solutions prepared by digesting 50 grams of straw, with and without added arsenic, are given in Tables 1 and 2.

DISCUSSION

Determinations were made on pure solutions of potassium arsenate, the Gutzeit evolution procedure being used, and the arsine was absorbed in sodium hypobromite in a Winkler spiral, as recommended by Robinson.[1] It was found necessary to use 10 cc. of a normal solution of the hypobromite in the spiral to obtain complete absorption, this being determined by placing a bit of Gutzeit paper in the outlet. Upon evaporation of this solution with sulfuric acid to decompose excess hypobromite a definite salt effect upon the color development was observed, dependent upon the quantity of sulfuric acid used, which was subsequently neutralized with sodium carbonate. The hypobromite was therefore discarded in favor of concentrated nitric acid saturated with bromine. With pure solutions of potassium arsenate, optimum (100%) recovery of 0.1 to 60 mg. of arsenic pentoxide was obtained. Evolution was from 100 cc. of solution, 5 per cent in sulfuric acid (about 1.8 N), to which 0.5 cc. of the stannous chloride solution, 1 cc. of potassium iodide solution and 8 grams of zinc had been added. A new lot of zinc was obtained, and the recovery dropped off to 70 per cent. The addition of 1 cc. of 10 per cent copper sulfate solution eliminated this difficulty.

For plant material straw chopped to 2 mm. in a Wiley mill was used. It was digested according to a necessary modification of the official method.[2] When the material was not thoroughly wet at the start spontaneous combustion occurred, and when acid was added to saturation, the contents frothed out of the flask. This material was apparently free of arsenic. Aliquots of the prepared solution were introduced into the evolution flask together with known quantities of arsenic. Recovery immediately dropped off, and no means could be found to bring it up to the 100 per cent obtained with pure solutions of potassium arsenate. Evolution in stages, increasing the amount of reagents, and a preliminary heating before evolution were tried to no avail, although the recovery of 0.2 mg. of arsenic pentoxide was brought to what was calculated as 96 per cent. At this point the arsenic trichloride distillation method was tried, and it was found that the material did contain arsenic to the extent of 0.6 p. p. m., or 0.01 mg. of arsenic pentoxide in the aliquot used. This reduced the recovery to 91 per cent. Further work on the arsine evolution method was then abandoned in favor of the trichloride distillation method, which proved to be much simpler and gave recoveries of

[1] Bur. Chem. and Soils Contact, **1**, No. 1, 20 (1931) and personal communication.
[2] *Methods of Analysis*, A.O.A.C., 1930, 307.

TABLE 1

Arsenic recovery by arsine evolution method

PURE SOLUTIONS			STRAW SOLUTIONS			REMARKS
As_2O_3 ADDED	RECOVERED	PER CENT	As_2O_3 ADDED	RECOVERED	PER CENT	
mg.	*mg.*		*mg.*	*mg.*		
0.200	0.200	100	0.200	0.155	77.5	Without $CuSO_4$
0.200	0.184	92	0.200	0.165	82.5	Without $CuSO_4$
0.200	0.210	105*	0.133	0.113	85.0	With $CuSO_4$
0.200	0.200	100	0.200	0.168	84.0	With $CuSO_4$
0.200	0.200	100	0.200	0.160	80.0	With $CuSO_4$
0.100	0.094	94	0.200	0.160	80.0	Evolution in 2 stages
0.200	0.140	70†	0.200	0.196	98.0	With preliminary heating
0.200	0.136	68	0.067	0.066	98.5	With preliminary heating
0.200	0.200	100	0.200	0.184	92.0	Boiled 10 min. before evolution
0.80	0.80	100	0.200	0.184	92.0	Boiled 10 min. before evolution
5.0	5.0	100	0.100	0.096	96.0	On steam bath 45 min. before evolution
10.0	10.2	102	4.0	3.68	92.0	On steam bath 45 min. before evolution
20.0	20.0	100‡	4.0	3.68	92.0	Same, 2 evolutions
40.0	39.2	98	0.217	0.194	89.4	Solution oxidized with $KMnO_4$ before evolution
60.0	61.2	102	0.000	0.000	—	Blank§
100.0	80.0	80				
0.000	0.000	—¶				

* Zn No. 1, without $CuSO_4$.
† Zn No. 2, without $CuSO_4$.
‡ Zn No. 2, with $CuSO_4$.
§ Blank.
¶ The straw did contain arsenic according to the arsenic trichloride distillation method.

TABLE 2

Arsenic recovery by arsenic trichloride distillation method

PURE SOLUTIONS

As₂O₃ ADDED	RECOVERED	REMARKS
mg.	mg.	
0.200	0.204	Distillation only
0.000	0.004*	Blank distillation
0.000	0.007*	Blank digestion and distillation

STRAW SOLUTIONS†

As₂O₃ ADDED	RECOVERED	NET RECOVERED	PER CENT
mg.	mg.	mg.	
0.000	0.035	—	—
0.200	0.240	0.205	102.5
0.200	0.240	0.205	102.5
1.00	1.02	0.985	98.5
5.00	5.00	4.96	99.2
10.0	10.0	9.96	99.6
10.0	10.2	10.16	101.6

* Unreadable in colorimeter; determined by comparison in 50 cc. flasks with flasks containing corresponding standards
† Arsenic added, prior to digestion, in the form of an aliquot of the standard solution.

100 per cent plus or minus an error of 2 per cent attributed largely to the colorimeter reading.

COMMENTS

The use of copper sulfate in the arsine evolution method is not always necessary as some supplies of zinc give a quantitative evolution of arsenic. The use of copper in these experiments does not confirm the results of Chouchak,[1] who obtained low results in its presence. However, it is believed best to use no more than enough copper to activate the zinc. The official methods state[2] that the zinc may be used again. Low results were obtained from pure solutions of arsenic when recovered zinc was used in this work.

In preparing the solution for the development of the color with the molybdenum blue reagent the final oxidation of the aliquot should be made with nitric acid, and the addition of the 1 per cent sulfuric acid should follow immediately. The development of the color should then be completed as rapidly as possible. Therefore, if the determination must be interrupted it would be best to discontinue it after the first evaporation.

In using the molybdenum blue reagent, the analyst should remember that it is a colloidal solution and that any dilution must be made before the development of the color.

Zinzadze states that the standards prepared with this reagent, if kept stoppered and in the dark, will not fade during a period of several days. The standards used in this work faded slightly after standing overnight, but this is attributed to impurities in the molybdenum trioxide, as a considerable blue color was obtained in the preparation of Solution I, whereas Zinzadze notes only a very slight blue in the preparation of this reagent. Therefore standards were prepared with each set of determinations.

It is proposed, in view of the difficulty of the digestion procedure, to adapt the dry ashing apparatus of Karns[3] to the determination of arsenic in plant materials by this method.

SUMMARY

(1) Recovery of arsenic from pure solutions is found to be complete when the arsine evolution method and Zinzadze's molybdenum blue reagent are used. This method, however, is not adapted to solutions obtained by the wet ashing of plant materials.

(2) When used with the arsenic trichloride distillation method this colorimetric method gives good recoveries from wet ashed samples.

(3) This method is applicable to quantities of arsenic pentoxide ranging from 0.002 mg. to 10.0 mg. Above 10.0 mg. dilution errors enter. As no more than 0.200 mg. in 50 cc. is readable, the corresponding dilution of 50 to 1 for 10.0 mg. reduces precision accordingly.

[1] *Ann. Chim. anal.*, 4, 2nd ser., 138 (1922).
[2] *Methods of Analysis*, A.O.A.C., 1930, 306.
[3] *J. Ind. Eng. Chem. Anal. Ed.*, 4, 299 (1932).

DETERMINATION OF AMMONIACAL NITROGEN IN FERTILIZERS WITHOUT DISTILLATION

By Z. I. KERTESZ, F. J. KOKOSKI and A. W. CLARK (New York State Agricultural Experiment Station, Geneva, N. Y.)

INTRODUCTION

The well-known reaction $(NH_4)_2SO_4 + 2NaOH = Na_2SO_4 + 2H_2O +2NH_3$ offers two possibilities for the determination of ammonia in ammoniates: (1) Distillation of the liberated ammonia and determination by direct titration in boric acid by Winkler's method[1] or by back titrating the excess of the acid when mineral acids are used, or (2) measurement of the quantity of alkali consumed in the liberation of ammonia. Although mentioned in some of the pioneer textbooks on analytical chemistry, such as those of Mohr[2] and Fresenius,[3] the second method has not come into general use and, so far as the writers are aware, is not mentioned in later textbooks on analytical chemistry.

In recent years several attempts have been made to obtain methods for the determination of ammonia without distillation. Manchot and Oberhauser[4] proposed a bromometric method for this purpose. Kolthoff and Laur[5] concluded that methods based on the oxidation of ammonia to nitrogen with hypobromite are not satisfactory because the reactions on which they are based are not quantitative. Auger[6] described a method for the titrametric determination of ammoniates in the presence of Nessler's reagent. Kertesz[7] published a preliminary paper describing a procedure for the determination of ammonia in ammoniates, especially in fertilizers, which is essentially the same as the method described in this paper. Recently, Sors[8] proposed the same method, but did not give details as to its applicability and limitations.

The purpose of this paper is to present definite directions for the determination of ammonia in ammoniates by the method first described by Mohr and later proposed by Kertesz and by Sors for the determination of ammonia in ammonium fertilizers.

EXPERIMENTAL

The method consists in driving off by boiling the ammonia liberated by added standard alkali and in titrating the excess of alkali with standard acid.

Preliminary determinations made to standardize the procedure showed that 0.5 N alkali (potassium hydroxide) and 0.1 N acid were best suited

[1] *Z. angew. Chem.*, **26**, 231 (1913).
[2] Lehrbuch der Chemisch-analytischen Titrirmethode, 4th ed. (1876).
[3] Quantitative Chemical Analysis, 7th ed. (1876).
[4] *Ber.*, **57**, 29 (1924).
[5] *Z. anal. Chem.*, **73**, 177 (1928).
[6] *Compt. rend.*, **178**, 1081 (1924).
[7] *Magyar Chem. Folyoirat*, **33**, 135 (1927).
[8] *Chem. Z.*, **56**, 156 (1932).

for these determinations. Aliquots of 25 cc. and 5 cc. of 0.5 *N* potassium hydroxide were used. Mixtures containing different quantities of ammoniates were boiled for different periods and titrated. It was found that the bulk of the ammonia was boiled off in less than 2 minutes and that the slowest part of the procedure was the boiling off of the final traces of ammonia, which cannot be detected by litmus paper as was proposed by Mohr.

In 210 seconds traces of ammonia were still present in the mixture, while in 240 seconds it was completely volatilized. However, in order to make sure that the ammonia is completely removed in all cases, 5 minutes of vigorous boiling is recommended when 30 cc. is used for the initial total volume.

During the five-minute period of boiling, the volume of the liquid diminishes to about 10 cc., while the concentration of salts in the solution increases proportionately, thus hastening the liberation of ammonia. When 5 grams of sodium chloride is added to the mixture, it is free of ammonia in 150 seconds.

The complete procedure used in these determinations of ammoniacal nitrogen in fertilizers was the following:

A 1 gram sample of the ammonium salt or ammonium fertilizer was leached with 250 cc. of cold water.* An aliquot of 25 cc. was placed in a 250 cc. extra wide-mouthed Erlenmeyer flask, and 5 cc. of 0.5 *N* potassium hydroxide was added. If the solution was not neutral, it was nearly neutralized while cold, methyl red being used as indicator; then the solution was boiled and neutralization was completed with 0.1 *N* acid or alkali solutions. (Alkaline solutions should not be warmed until slightly acid to methyl red.) A faint red color was taken for the end point in all cases (standardization, initial neutralization, final titration). Special care must be taken to have exactly the same end point throughout the entire procedure in the presence of phosphates in view of the buffer properties of the solutions. After the addition of the alkali, the solution was boiled vigorously for 5 minutes. The rate of boiling should be such that at the end of the 5 minutes the volume of the liquid which was originally 30 cc. should be about 10 cc., but precaution should be taken to prevent evaporation to dryness. The solution was boiled, then diluted to 50 cc. with freshly boiled distilled water, heated to boiling, and titrated with 0.1 *N* hydrochloric acid. One cc. of normal alkali is equivalent to 0.01401 gram of ammoniacal nitrogen.

In Table 1 are presented the results obtained with the ordinary grade of C. P. chemicals. No attempt was made to purify or dry these salts. The two sets of results given under the heading represent two separate leach-

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* The writers selected as a standard 1 gram leached to 250 cc. because it is the same as the present leaching used for the determination of insoluble phosphoric acid. They wish to note, however, that the available information as to the applicability of the present method for mixed fertilizers is not sufficient to decide this point.

TABLE 1

Ammonia in C.P. chemicals

(Percentage of ammoniacal nitrogen)

NO.	SAMPLE	BY DISTILLATION				PROPOSED METHOD		THEORETICAL
		WITH MgO (OFFICIAL)	Average	WITH NaOH	Average		Grand Average	
1	Ammonium sulfate (NH₄)₂SO₄	20.80 20.98 —	20.89	21.12 20.92	21.02	20.97 20.95 21.06 20.97 Av.: 20.99 21.08 20.93 21.00 20.96 Av.: 20.99	20.99	21.21
2	Ammonium Nitrate NH₄NO₃	17.34 17.24 —	17.29	17.32 17.26	17.29	17.49 17.42 17.42 17.38 Av.: 17.42 17.47 17.43 17.57 17.57 Av.: 17.51	17.47	17.50
3	Ammonium Chloride NH₄Cl	25.73 24.91 24.10 25.74	25.12	25.92 26.22	26.07	26.31 26.13 26.16 26.18 Av.: 26.19 26.13 26.16 26.17 Av.: 26.14	26.16	26.19
4	Di-ammonium Phosphate (NH₄)₂HPO₄	20.24 20.67 20.84 20.65	20.60	20.98 20.96	20.97	20.79 20.88 20.86 20.81	20.83	21.21

TABLE 2

Ammonia in ammonium sulfate fertilizers

(Percentage of ammoniacal nitrogen)

| NO. | SAMPLE | BY DISTILLATION | | | | | | | | PROPOSED METHOD | | | Grand Average |
		WITH MgO (OFFICIAL)				Average	WITH NaOH		Average				
1	750	20.31	20.59	20.63	—	20.51	20.92	20.94	20.93	20.97 20.90	20.83 21.01	Av.: 20.90 Av.: 20.95	20.93
2	692	20.86	20.86	—	—	20.86	20.88	20.80	20.84	20.97 20.90	20.93 21.02	Av.: 20.95 Av.: 20.96	20.96
3	745	19.86	20.56	20.45	20.59	20.39	20.60	—	20.60	20.54 20.63	20.61 20.70	Av.: 20.58 Av.: 20.67	20.63
4	662	20.13	21.03	19.88	20.56	20.40	20.92	—	20.92	20.83	20.76	—	20.80
5	754	20.74	21.10	20.84	20.76	20.86	20.86	—	20.86	20.90	20.85	—	20.88
6	210	19.59	19.53	20.62	—	19.91	20.74	—	20.74	20.64	20.69	—	20.67

TABLE 3
Ammonia in other ammonium fertilizers
(Percentage of ammoniacal nitrogen)

NO.	SAMPLE	BY DISTILLATION								PROPOSED METHOD					GRAND AVERAGE
		WITH MgO (OFFICIAL)				Average	WITH NaOH		Average						*Grand Average*
1	578 (Ammo-phos)	10.37	10.49	10.34	10.48	10.42	10.97	11.00	10.99	10.68	10.64	10.48	10.55	Av.: 10.59	10.64
										10.60	10.77	10.67	10.68	Av.: 10.67	
2	AZ (Cal-nitro)	7.92	8.14	—	—	8.04	—	—	—	8.31	8.27	8.62	8.58	Av.: 8.44	8.49
										8.58	8.55	8.48	8.52	Av.: 8.53	
3	LP (Leuna-phos)	19.62	19.74	—	—	19.71	—	—	—	19.87	19.66	19.77		Av.: 19.77	19.79
										19.80	19.80	19.80		Av.: 19.80	
4	B (Ammo-phos B)	16.65	16.56	—	—	16.61	—	—	—	16.44	16.47	16.46		Av.: 16.46	16.45
										16.57	16.32	16.44		Av.: 16.44	
5	2A (Ammo-phos-ko)	8.82	8.90	—	—	8.86	—	—	—	9.37	9.37	9.37		Av.: 9.37	9.34
										9.37	9.23	9.30		Av.: 9.30	

ings. In the case of the determinations by distillation, with the exception of some determinations of the lot presented in Table 4, the salts were used without previous leaching.

The results obtained on pure salts by the different methods do not always agree. The results obtained by the three different methods with ammonium sulfate are comparable, but all results are lower than the theoretical. The low results may be attributable to impurities or moisture. In ammonium nitrate the results obtained by the proposed method are higher by about 0.2 per cent nitrogen than the results obtained by distillation. In this case, however, as also in the case of ammonium chloride, the results obtained by the method presented agree well with the theoretical nitrogen content. In di-ammonium phosphate, $(NH_4)_2HPO_4$, all determinations gave results lower than the theoretical nitrogen content. The variation between individual determinations by the proposed method was smaller than that between corresponding determinations by the other methods. Attention is called to the variation in results obtained by the official method.

There is often some organic material in ammonium sulfate fertilizers, most of which is retained by the filter paper when the sample is leached. Nevertheless some organic materials might go into solution and interfere with the results. It was considered important, therefore, to get an insight into the effects of these materials on the results obtained by the method without distillation. The results presented in Table 4 indicate that many of the organic materials common in fertilizers do not affect the ammonia determination and that the urea is not affected by the relatively short heating with alkali of an initial concentration of less than normal. The results presented in Table 4 marked by a star were derived from the material which was leached according to the original directions. The results obtained by the official method, by the proposed method, and also by the sodium hydroxide distillation when leachings were used, agree well, except in the first three samples. These were urea and two samples of cyanamide. The results obtained by sodium hydroxide distillation on the unleached and leached samples indicate the necessity of using leaching with the method proposed because organic materials are not only liable to give off ammonia when boiled with alkali, but they also may consume alkali for saponification, etc. This danger is not entirely obviated by filtration, but at least the majority of the organic impurities are removed by this procedure.

SUMMARY

Ammonia in pure ammonium salts and ammonium fertilizers may be determined without distillation by replacing the ammonium in the salts by alkali metals. The ammonia replaced by the alkali is driven off

TABLE 4

Determinations on organic materials

(Percentage of ammoniacal nitrogen)

NO.	SAMPLE	BY DISTILLATION								PROPOSED METHOD				
		WITH MgO (OFFICIAL)				Average	WITH NaOH		Average					Average
1	Urea†	2.52	3.32	—	—	—	5.32	5.34	5.33	0.67	0.46	0.68	0.63	0.61
2	1003 (Cyanamide)	1.29	1.25	1.49	0.75	—	—	—	—	0.32	0.38	0.46	0.25	0.35
3	354 (Cyanamide)	1.31	1.30	1.32	1.50	1.36	—	—	—	0.00	0.00	—	—	0.00
4	227 Millorganite	0.10	0.12	—	—	0.11	0.20*	0.20*	0.20*	0.24	0.20	—	—	0.22
5	1 Bone	0.11	0.11	—	—	0.11	0.43	0.44	0.44	0.10	0.10	—	—	0.10
							0.20*	0.12*	0.16*					
6	721 Castor pomace	0.09	0.09	—	—	0.09	0.00*	0.04*	0.02*	0.10	0.10	—	—	0.10
7	92 Fish Meal	0.19	0.18	—	—	0.19	1.28	1.42	1.35	0.20	0.20	—	—	0.20
							0.16*	0.20*	0.18*					
8	739 Cottonseed meal	0.12	0.11	—	—	0.12	0.08*	0.08*	0.08*	0.10	0.10	—	—	0.10
9	566 Tankage	0.34	0.36	—	—	0.35	1.19	1.11	1.15	0.50	0.30	—	—	0.40
							0.28*	0.32*	0.30					
10	25 Dried Blood	0.46	0.32	—	—	0.39	1.24	1.38	1.31	0.30	0.20	—	—	0.25
							0.36*	0.32*	0.34*					

† 46 per cent total nitrogen.
* Leached.

in a short time by boiling, and the excess of alkali is then measured by titration with standard acid.

This method gives good results on pure ammonium salts and ammonium sulfate fertilizers. The results on other ammonium fertilizers are somewhat more divergent than those obtained on ammonium sulfate.

The chief advantage of the method is that results may be obtained in much shorter time than by the usual distillation method.

DETERMINATION OF CALCIUM IN MINERAL MIXTURES

By V. W. MELOCHE, Chemistry Department, University of Wisconsin; and L. E. CLIFCORN and W. B. GRIEM,[1] Wisconsin Feed and Fertilizer Inspection Laboratory

The determination of calcium in mineral feeds is complicated by the presence of phosphorus, magnesium, iron, and aluminum, and most of the existing methods are too long for rapid routine work. Several of the shorter methods proposed include a single precipitation of calcium oxalate and subsequent titration of the oxalate with potassium permanganate. The results obtained, however, are often inaccurate. The purpose of the investigation described was to select a rapid procedure in which the conditions of precipitation of pure calcium oxalate are more certainly controlled.

When a parallel study was made of the existing short methods for the determination of calcium under the conditions described in the literature for the successful precipitation of pure calcium oxalate, the accuracy of the present tentative A.O.A.C. method[2] was questioned in regard to the precipitation of calcium oxalate in the presence of phosphorus at a pH of 5 by means of ammonium oxalate. Because the addition of the first few drops of ammonium oxalate caused the methyl red indicator to change from brown to yellow, it seemed probable that a considerable portion of the calcium oxalate was precipitated in a very weakly acid medium, and therefore contamination would be expected when the precipitation was made in the presence of phosphorus.

Studies by Meade,[3] Passon,[4] McCrudden,[5] Winkler,[6] Breazeale,[7] Kramer and Howland,[8] and others, as well as the work of investigators in this laboratory, indicate that the precipitation of calcium as oxalate in the presence of phosphate should take place in relatively strong acid solution.

[1] Director.
[2] *Methods of Analysis*, A.O.A.C., 1930, 3, 287.
[3] *Chem. Eng.*, 1, 21 (1895).
[4] *Z. angew. Chem.*, 11, 776 (1898); 12, 48 (1899); 14, 285 (1901).
[5] *J. Biol. Chem.*, 7, 83 (1910); 10, 187 (1912)
[6] *Z. angew. Chem.*, 31, I, 214 (1918).
[7] *This Journal*, 4, 124 (1921).
[8] *J. Biol. Chem.*, 68, 711 (1926).

Shohl[1] recommends a specific pH range of 4–5.6. Chapman[2] shows that quantitative precipitation is obtained in solutions as acid as pH 3.8 and at pH 2.2 the precipitation of calcium oxalate is almost complete (319 parts out of 321). At pH 2.2 he could not detect the interference of reasonable quantities of manganese, iron, aluminum and magnesium. Richards[3] and Blasdale[4] show that the occlusion of magnesium oxalate, at the time of precipitation of calcium oxalate, is greatly decreased by precipitation in strongly acid solution. Popoff, Waldbauer, and McCann[5] verified this finding and showed that digestion of the precipitate is an important factor, that enough oxalate must be present for both calcium and magnesium, and that the addition of ammonium chloride is unnecessary.

EXPERIMENTAL

The following procedure was designed to cause precipitation of calcium oxalate in such a manner that upon addition of the precipitant the greater part of the calcium oxalate is formed in a strongly acid medium (pH 2).

PROPOSED METHOD

REAGENTS

Oxalic acid solution.—2.5 per cent.
Ammonium oxalate solution.—3 per cent.
Sodium acetate solution.—20 per cent.
Aqua ammonia.—36 cc. of concentrated NH_4OH (sp. gr. 0.89) per liter.
Potassium permanganate solution.—0.1 N.

PROCEDURE

Weigh a 2 gram sample of the finely ground material into a small evaporating dish and ignite to a carbon-free ash (taking care that none of the sample is lost by decrepitation). Dissolve the ash in hydrochloric acid (1+3), finally heating to insure complete solution. Filter into a 250 cc. volumetric flask, dilute to the mark, mix thoroughly, and pipet 25 cc. into a 400 cc. beaker. Using methyl red as the indicator, neutralize with ammonia, and then make the solution distinctly acid by the addition of hydrochloric acid (1+4). Dilute to 250 cc., add 20 cc. of the oxalic acid solution and heat to boiling. Digest at the boiling temperature for 30 minutes, stirring occasionally. Add 20 cc. of the ammonium oxalate solution and digest for 15 minutes; then add slowly 10 cc. of the sodium acetate solution, cool, and allow to stand at least 4 hours (no longer if magnesium content of sample is high). Filter and wash the precipitate, using the following technic: Decant off the supernatant liquid through a Gooch crucible and wash twice by decantation with 75 cc. portions of the aqua ammonia solution; transfer the precipitate to the crucible and wash with 150 cc. more of the 1 per cent ammonia. Place the crucible and contents in the beaker and add 250 cc. of distilled water and 10 cc. of sulfuric acid. Titrate slowly with the potassium permanganate solution, which has been standardized against pure sodium oxalate or calcite of known composition. Correct for the blank and calculate the percentage of calcium oxide.

[1] *J. Biol. Chem.*, **50**, 527 (1922).
[2] *Soil Sci.*, **26**, 479 (1928).
[3] *Proc. Am. Acad. Arts and Sci.*, **36**, 392 (1900–1901).
[4] *J. Am. Chem. Soc.*, **31**, 917 (1909).
[5] *J. Ind. Eng. Chem. Anal. Ed.*, **4**, 1, 43 (1932).

The hydrogen-ion concentrations at different stages during the precipitation, followed by means of the quinhydrone electrode, are shown in Table 1.

TABLE 1

Hydrogen-ion concentration

	25°C.
250 cc. solution distinctly acid to methyl red.	$pH = 4.15$
Same as above plus 20 cc. of 2.5 per cent oxalic acid*.	$pH = 2.00$
Same as above plus 20 cc. of 3 per cent ammonium oxalate. . .	$pH = 3.35$
Same as above, heated to boiling and 10 cc. of 20 per cent sodium acetate added—cooled.	$pH = 4.50$

* The greater portion of the calcium oxalate is precipitated during the digestion following this addition of oxalic acid.

The proposed method and the present tentative A.O.A.C. method were tested on samples of known quantities of calcium in the presence of phosphorus, magnesium, and iron.

Standard calcium chloride solution.—8.0690 grams of pure calcite was dissolved in hydrochloric acid and diluted to 2 liters; 25 cc. of this solution was equivalent to 0.0565 gram of calcium oxide.

Dilute ortho phosphoric acid solution.—1 cc. was equivalent to 0.01152 gram of phosphorus pentoxide.

Dilute magnesium chloride solution.—1 cc. was equivalent to 0.00716 gram of magnesium oxide.

Dilute ferric chloride solution.—1 cc. was equivalent to 0.01454 gram of ferric oxide.

The phosphorus, magnesium, and iron solutions were tested and found to be free from calcium.

In trials with the A.O.A.C. method the precipitate was filtered on a Gooch crucible and washed as in the proposed method. The results, which are averages of duplicate determinations, are recorded in Table 2. The percentages of calcium oxide recovered, assuming an aliquot as representing a 0.2 gram sample, are also given.

To point out a comparison between the two methods on a practical basis for the analysis of phosphatic materials, samples of C.P. tri-calcium phosphate and C.P. di-calcium phosphate were analyzed. A comparison was also made on a high magnesium sample of dolomitic limestone. The samples were analyzed volumetrically by double precipitation, the proposed method being used to give the correct results for calcium oxide. The percentages of phosphorus pentoxide on the phosphate samples were determined by the A.O.A.C. method used on fertilizers. The percentage of magnesium oxide in the dolomitic limestone was determined on the combined filtrates of the double precipitation of the calcium by precipitating magnesium ammonium phosphate and determining as magnesium pyrophosphate. Averages of duplicate determinations are recorded in Table 3.

TABLE 2

Comparison of A. O. A. C. and proposed method

CaO TAKEN		A. O. A. C. METHOD			PROPOSED METHOD		
		CaO RECOVERED		ERROR	CaO RECOVERED		ERROR
gram	*per cent*	*gram*	*per cent*	*per cent*	*gram*	*per cent*	*per cent*
0.05650*	28.25	0.05651	28.26	+0.01	0.05655	28.28	+0.03
0.05650* 0.01152 †	28.25	0.05639	28.20	−0.05	0.05648	28.24	−0.01
0.05650* 0.02304 †	28.25	0.05605	28.03	−0.22	0.05644	28.22	−0.03
0.05650* 0.03456†	28.25	0.05620	28.10	−0.15	0.05633	28.17	−0.08
0.05650* 0.04608 †	28.25	0.05591	27.96	−0.29	0.05637	28.19	−0.06
0.05650* 0.06912 †	28.25	0.05563	27.82	−0.43	0.05645	28.23	−0.02
0.05650* 0.09216 †	28.25	0.05546	27.73	−0.52	0.05644	28.22	−0.03
0.1130* 0.13824 †	56.50	0.11162	55.81	−0.69	0.11342	56.71	+0.21
0.05650* 0.02148 †	28.25	.05665	28.33	+0.08	0.05647	28.24	−0.01
0.05650* 0.03580‡	28.25	0.05693	28.46	+0.21	0.05671	28.36	+0.11
0.05650* 0.05020‡	28.25	0.05690	28.45	+0.20	0.05653	28.27	+0.02
0.05650* 0.05728‡	28.25	0.05676	28.38	+0.13	0.05683	28.42	+0.17
0.05650* 0.07160‡	28.25	0.05715	28.58	+0.33	0.05679	28.40	+0.15
0.05650* 0.01454§	28.25	0.05642	28.21	−0.04	0.05669	28.35	+0.10
0.05650* 0.04362§	28.25	0.05591	27.96	−0.29	0.05661	28.31	+0.06
0.05650* 0.07270§	28.25	0.05600	28.00	−0.25	0.05702	28.51	+0.26

* CaO.   † P₂O₅.   ‡ MgO.   § Fe₂O₃.

DISCUSSION

From a study of Tables 2 and 3 it is obvious that the proposed method has the desired accuracy for the analysis of mineral mixtures and ingredients in which there are varying quantities of phosphorus, magnesium, and iron. The conditions for quantitative precipitation of calcium oxalate in the presence of phosphorus and iron have been controlled by the high acidity in which the precipitation is made. The conditions for magnesium have been controlled by the high acidity, digestion of the sample, and filtration as soon as possible after the precipitation. Digestion

TABLE 3

Averages of duplicate determinations of calcium oxide

	CORRECT COMPOSITION	A.O.A.C. METHOD	ERROR	PROPOSED METHOD	ERROR
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
$Ca_3(PO_4)_2$	48.53 CaO	47.93	−0.60	48.70	+0.17
	42.98 P_2O_5				
CaH PO$_4$	32.85 CaO	32.29	−0.56	32.93	+0.08
	41.33 P_2O_5				
Dolomite	30.38 CaO	30.39	+0.01	30.32	−0.06
	23.35 MgO				

of the precipitate in acid solution increases the size of the particles, and thus reduces the total surface of the precipitate. This causes the calcium oxalate formed to filter and wash easily and have a low solubility in the wash solution. The earliest possible filtration of the calcium oxalate from the precipitating medium is recommended in samples containing a high magnesium content, since magnesium oxalate has the tendency to precipitate out slowly on standing.

By filtering the calcium oxalate on a Gooch crucible the error in titration caused by the action of potassium permanganate on the filter paper has been avoided.[1]

The solubility of calcium oxalate in the water used to wash the precipitate is great enough to cause error. The low solubility of calcium oxalate in dilute ammonia has enabled various investigators to use it successfully as a wash solution.[2] In the proposed method 1 per cent aqua ammonia gives satisfactory results as a wash solution.

The low results obtained by the use of the A.O.A.C. method in the presence of phosphate are explained by the precipitation of calcium oxalate at a hydrogen-ion concentration which permits the co-precipitation of calcium phosphate.

[1] *J. Am. Chem. Soc.*, **39**, 928 (1917).
[2] *J. Biol. Chem.*, **63**, 461 (1925).

SUMMARY

An accurate method for the analysis of calcium in mineral mixtures and their ingredients is proposed. Its accuracy was compared to that of the present tentative A.O.A.C. method on known samples of calcium in the presence of phosphorus, magnesium, and iron. From a comparison of the two methods, *especially in the presence of phosphorus*, it is obvious that greater accuracy is obtained by the proposed method in which the initial precipitation of calcium oxalate takes place in a strongly acid solution.

QUALITATIVE DETECTION OF LEAD IN SPRAY RESIDUES[1]

By M. HARRIS (U. S. Food and Drug Administration, Chicago, Ill.)

Directions for the microchemical detection of lead based on the hexanitrite reaction, for spot tests depending on different colors obtained with various organic reagents, and for microelectrolytic separation as peroxide or as metallic lead are extensively described in the literature.

The emergent need of a quick qualitative test prohibited a thorough investigation of the above-mentioned procedures. A spot test[2] based on the reaction of a 0.5 per cent ammoniacal solution of carminic acid with a nearly neutral lead solution was tried with negative results. An alternative test involving the use of a dilute pyridine solution followed by a 0.1 per cent solution of gallocyanine also proved unsuccessful.

The proposed method is based on the turbidity formed on addition of sodium bisulfite to a slightly acid or neutral solution of lead, the principle utilized in the nephelometric estimation of minute quantities of lead. A positive test was obtained with 0.1 mg. of lead oxide when the method outlined below was used. However as a matter of precaution 0.15 mg. was used. To obviate the chance of false conclusions which might be caused by the presence of barium and strontium, the presence of lead was confirmed by conversion of the sulfite to the iodide by means of a potassium bromide-potassium iodide solution and subsequent microscopic identification[3] of the lead iodide.

When the procedure outlined was applied to a dilute alkali wash solution obtained in the alkali quick method[4] for the determination of arsenic in apples sprayed with lead arsenate it was observed that the presence of foreign and organic matter interfered with the formation of the turbidity and lead iodide plates. This difficulty was eliminated by evaporating the wash solution to dryness, leaching out the lead salt with hot water, and filtering. The bisulfite was then added to the clear filtrate. The amount of wash solution that would contain about 0.15 mg. of lead oxide

[1] Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., November, 1932.
[2] *Mikrochemie*, 7, 301 (1929).
[3] *Bull. Soc. Chim.*, (IV) 45, 678 (1929).
[4] Unpublished.

was estimated from the previously determined arsenic value by assuming the ratio of PbO to As$_2$O$_3$ in arsenical spray residue to be 2.5 to 1.

The details of the proposed method are as follows:

<div align="center">REAGENTS</div>

(a) *Sodium bisulfite.*—C.P. powdered.

(b) *Nitric acid.*—(1+5).

(c) *Potassium iodide—potassium bromide solution.*—Dissolve 12.5 gram of KI and 12.5 gram of KBr in 50 cc. of water.

(d) *Nitric acid.*—(1+13).

(e) *Ammonia.*—(1+2).

<div align="center">DETERMINATION</div>

Withdraw from the clear diluted alkali wash solution obtained in the alkali quick method for arsenic a sufficient quantity to contain about 0.15 mg. or more of lead oxide. The following formula will be of assistance in calculating from the arsenic content the minimum number of cc. to be used.

If a = aliquot in cc. taken for arsenic determination, and
b = As$_2$O$_3$ in mg. found in a,

then the quantity of solution containing about 0.15 mg. of lead oxide may be expressed by "x" in the following equation:

$$x = \frac{0.06a}{b}.$$

Make the solution slightly acid to litmus paper with dilute nitric acid (reagent b), and evaporate just to dryness on the hot plate or over a low flame. Take up the residue in 5 cc. of hot water, stir thoroughly, and filter (CS & S No. 589 Blue Ribbon—9 cm.), receiving the filtrate in a test tube (125 mm. ×15 mm.). Add dilute ammonia dropwise until practically all excess acid is neutralized (just slightly acid to litmus). Add 0.1–0.2 gram of sodium bisulfite, and agitate to bring into solution. If a turbidity develops in a few minutes, lead is indicated.

To confirm the presence of lead, centrifugalize the material, carefully decant all the supernatant liquid, and add 2–3 drops of the dilute nitric acid (reagent d) to the precipitate. When the precipitate is completely dissolved, transfer a drop of the solution onto a slide and carefully place alongside it a drop of the potassium iodide-potassium bromide solution. Combine both drops by means of a platinum wire and examine the precipitate microscopically. If the crystals first formed should redissolve, add to the slide another drop of the solution in the test tube. Lead iodide forms well-defined hexagonal plates which are brownish-yellow by transmitted light. By reflected light lead iodide plates glisten and display the iridescent colors of thin films, a characteristic feature of this salt.

To produce known lead iodide crystals for comparison use 1 drop of lead acetate solution prepared as follows: Weigh out 15 mg. of normal lead acetate, add one drop of dilute nitric acid (1+3), and make up to 30 cc. with distilled water.

The above method was applied to the following mixtures with the results indicated:

(1) Alkali wash solution (50 cc.) from apples containing no lead . . . . Negative

(2) Wash solution (1) to which was added 0.15 mg. of PbO . . . . Positive

(3) Solution (1) to which was added soluble salts of calcium, copper, arsenic, barium, iron, sodium silico fluoride, magnesium, chlorides, and fluorine . . . . Negative

(4) Solution (3), 50 cc., plus 0.15 mg. of PbO . . . . Positive

THE COMPOSITION OF WHITES, YOLKS, AND WHOLE EGGS BROKEN OUT BY COMMERCIAL EGG-BREAKING ESTABLISHMENTS

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The composition of eggs broken out by six firms engaged in the manufacture of frozen yolks, whites, and whole eggs is reported in this paper. Mitchell[3] reported the composition of shell eggs broken out in the laboratory, the separation of yolks and whites and removal of adhering white from the yolk and the shell being made as complete as possible. In the commercial production of frozen and liquid yolks it is not practical to effect as complete a separation of yolk and white as in the laboratory, nor can the white be as completely removed from the shell. Commercially produced liquid or frozen eggs, therefore, will contain more white in the yolk and less white in the whole eggs than those broken out and carefully separated in the laboratory. The differences between the commercial and laboratory product make this paper of interest.

Arrangements were made with three firms of egg breakers in Chicago and three in St. Louis to break out random samples of eggs from time to time from the beginning to the end of the season in the spring of 1932. The breakers were instructed to make as clean a separation of yolk and white as commercially possible, and to break out the whole eggs so as to leave the minimum of white adhering to the shell.

The samples of white, yolk, and whole egg were then analyzed according to the methods previously given by Mitchell.[3] The results are given in Tables 1 and 2. Tables 3 and 4 give the results calculated to the dry basis. Samples with the prefix "C" were broken out and analyzed in Chicago, those with "S" in St. Louis. The sample of whole egg is from the same shell egg stock as the corresponding yolk and white and bears the same number with the suffix "W". The column headed "Source" indicates the state of production of the shell egg stock. All stock was from 3 to 16 days old.

TABLE 1

Composition of commercially separated yolks and whites
(Results are expressed in percentage)

SAMPLE NO.	SOURCE	DATE COLLECTED	MFGR.	WHITES			YOLKS				
				SOLIDS	TOTAL N	WATER-SOL. N	SOLIDS	FAT	P_2O_5	TOTAL N.	WATER-SOL. N.
		1932									
C-1	Mo.	3/22	CA	12.21	1.74	1.63	46.75	28.42	1.22	2.56	0.64
C-2	Ill.	3/28	CB	12.36	1.75	1.66	45.57	27.75	1.20	2.46	0.62
C-3	Wis.	3/31	CA	12.27	1.77	1.63	46.78	28.57	1.27	2.57	0.61
C-4	Mo.	4/4	CB	11.74	1.68	1.55	45.21	27.33	1.19	2.50	0.62
C-5	Ill.	4/4	CC	12.63	1.81	1.66	47.33	29.54	1.27	2.55	0.57
S-1	Mo.	4/5	SA	12.52	1.80	1.65	47.11	29.07	1.28	2.58	0.57
C-6	Ia.	4/7	CA	12.24	1.75	1.61	47.32	29.18	1.28	2.54	0.57
C-7	Neb.	4/7	CC	12.07	1.69	1.59	47.08	29.09	1.27	2.57	0.59
C-8	Ill.	4/11	CB	12.29	1.75	1.65	46.25	28.60	1.26	2.52	0.58
C-9	*	4/11	CB	12.15	1.71	1.63	44.96	27.47	1.19	2.55	0.62
S-3	Mo.	4/12	SA	12.36	1.76	1.67	46.80	28.96	1.26	2.54	0.58
C-10	Mo.	4/14	CA	12.62	1.80	1.69	44.97	27.22	1.20	2.51	0.64
C-11	Ill.	4/14	CC	12.71	1.80	1.70	45.39	27.40	1.19	2.53	0.65
C-12	Minn.	4/18	CB	12.41	1.81	1.69	45.50	27.94	1.24	2.50	0.62
C-13	Ill.	4/18	CB	12.40	1.79	1.66	45.90	28.24	1.29	2.52	0.61
S-5	Mo.	4/20	SA	12.38	1.73	1.64	47.17	28.84	1.28	2.52	0.57
S-6	Mo.	4/20	SB	11.95	1.66	1.56	45.62	27.62	1.23	2.49	0.60
C-14	Ia.	4/21	CA	12.41	1.78	1.68	44.94	27.24	1.20	2.55	0.60
C-15	Ia.	4/21	CC	12.40	1.80	1.67	45.52	27.84	1.23	2.51	0.61
C-16	Minn.	4/25	CB	12.21	1.74	1.64	43.68	26.34	1.14	2.51	0.66
C-17	Ind.	4/25	CB	12.23	1.75	1.63	45.57	28.19	1.23	2.52	0.62
S-7	Ill.	4/27	SA	12.46	1.72	1.61	47.17	28.92	1.29	2.57	0.59
S-8	Mo.	4/27	SB	12.60	1.74	1.63	46.12	28.00	1.27	2.52	0.61
S-9	Mo.	4/28	SC	12.47	1.75	1.65	46.38	28.51	1.28	2.53	0.59
C-18	Mo.	4/28	CA	12.57	1.77	1.67	45.28	27.65	1.25	2.47	0.63
C-19	Okla.	4/28	CC	12.50	1.76	1.65	46.39	28.78	1.26	2.48	0.59
S-10	Ill.	4/29	SA	12.07	1.69	1.60	47.51	29.31	1.29	2.55	0.57
C-20	Ill.	5/2	CB	12.32	1.75	1.64	44.85	27.57	1.23	2.46	0.61
C-21	Minn.	5/2	CB	12.41	1.76	1.63	44.56	26.87	1.21	2.49	0.61
S-11	Mo.	5/3	SB	12.68	1.78	1.67	46.19	27.79	1.26	2.52	0.61
S-12	Mo.	5/3	SC	11.69	1.64	1.54	48.06	29.44	1.29	2.56	0.56
S-13	Mo.	5/5	SA	12.03	1.64	1.53	47.72	29.41	1.29	2.48	0.53
S-14	Mo.	5/5	SB	12.33	1.69	1.60	45.91	28.26	1.27	2.49	0.56
C-22	Okla.	5/5	CA	12.22	1.74	1.60	44.77	27.06	1.24	2.49	0.60
C-23	Okla.	5/5	CC	12.33	1.75	1.63	45.88	28.10	1.28	2.50	0.58
C-24	Ill.	5/9	CB	11.95	1.69	1.59	45.44	27.95	1.23	2.49	0.60
C-25	Wis.	5/9	CB	12.30	1.75	1.65	45.33	27.62	1.19	2.53	0.61
S-15	Ill.	5/9	SA	12.29	1.71	1.60	47.07	29.01	1.28	2.51	0.55
S-16	Mo.	5/9	SB	12.33	1.71	1.61	47.05	29.12	1.28	2.50	0.55
S-17	Mo.	5/11	SA	12.84	1.78	1.67	46.18	28.14	1.27	2.50	0.59
S-18	Mo.	5/11	SB	12.35	1.73	1.61	44.51	26.64	1.21	2.49	0.63
S-19	Kan.	5/13	SA	12.28	1.75	1.63	45.66	27.87	1.25	2.49	0.58
S-20	Mo.	5/13	SB	12.49	1.78	1.66	47.00	28.99	1.28	2.54	0.55

* Mixed lot.

TABLE 1 (*Continued*)

SAMPLE NO.	SOURCE	DATE COLLECTED	MFGR.	WHITES			YOLKS				
				SOLIDS	TOTAL N.	WATER-SOL. N.	SOLIDS	FAT	P₂O₅	TOTAL N.	WATER-SOL.N.
C-26	Ill.	5/16	CB	12.33	1.78	1.63	45.00	27.53	1.19	2.47	0.61
C-27	Wis.	5/16	CB	12.20	1.75	1.63	45.81	28.22	1.24	2.50	0.58
S-21	Ill.	5/17	SA	12.30	1.76	1.64	46.50	28.32	1.27	2.52	0.58
S-22	Mo.	5/17	SB	12.35	1.75	1.63	45.60	27.80	1.25	2.49	0.60
C-28	Ill.	5/19	CA	12.10	1.71	1.62	45.86	28.15	1.28	2.51	0.59
C-29	Okla.	5/19	CC	12.41	1.76	1.63	46.28	28.45	1.27	2.51	0.55
S-23	Ill.	5/20	SA	12.67	1.80	1.69	47.18	28.78	1.28	2.52	0.59
S-24	?	5/20	SB	12.08	1.70	1.60	43.86	26.58	1.20	2.46	0.67
C-30	Minn.	5/23	CB	12.11	1.71	1.60	45.99	28.01	1.24	2.50	0.60
S-25	Ill.	5/24	SA	12.36	1.75	1.64	47.10	28.89	1.27	2.52	0.58
S-26	Mo.	5/24	SB	12.54	1.77	1.67	45.65	27.60	1.24	2.50	0.63
C-31	Mo.	5/25	CA	12.71	1.70	1.63	45.81	28.19	1.25	2.47	0.57
C-32	Mo.	5/25	CC	12.91	1.85	1.71	46.49	29.00	1.24	2.50	0.56
S-27	Ill.	5/26	SA	12.64	1.80	1.68	46.95	28.66	1.28	2.56	0.60
S-28	Mo.	5/26	SB	12.47	1.77	1.66	44.42	26.60	1.20	2.53	0.67
S-29	Ill.	6/1	SA	12.78	1.81	1.70	45.94	27.64	1.25	2.52	0.62
S-30	Mo.	6/1	SB	12.46	1.74	1.64	45.45	27.44	1.24	2.52	0.61
C-33	Wis.	6/2	CA	12.14	1.69	1.59	43.54	26.10	1.21	2.49	0.65
C-34	Mo.	6/2	CC	12.42	1.72	1.63	46.43	28.66	1.29	2.52	0.56
S-31	Mo.	6/3	SA	12.07	1.72	1.60	47.28	29.23	1.30	2.53	0.58
S-32	Kan.	6/3	SB	12.25	1.73	1.63	44.74	27.13	1.21	2.47	0.64
C-35	Ill.	6/6	CB	12.13	1.70	1.62	46.45	28.44	1.26	2.54	0.56
C-36	Ill.	6/6	CB	12.39	1.73	1.63	46.66	28.95	1.28	2.48	0.56
C-37	Mo.	6/16	CA	12.43	1.75	1.65	43.44	26.47	1.16	2.40	0.62
C-38	Ia.	6/16	CC	12.55	1.78	1.64	46.31	28.31	1.27	2.55	0.57
C-39	Ill.	6/23	CB	12.58	1.78	1.68	45.13	27.62	1.20	2.49	0.58
C-40	Wis.	6/23	CB	12.14	1.73	1.62	44.36	26.55	1.18	2.50	0.66
C-41	Ia.	6/30	CA	11.91	1.64	1.58	43.48	26.27	1.17	2.40	0.66
C-42	Okla.	6/30	CC	12.47	1.75	1.66	45.70	28.03	1.22	2.51	0.57
C-43	Minn.	7/18	CB	11.95	1.68	1.55	44.54	27.41	1.23	2.42	0.60
C-44	Neb.	7/18	CC	12.41	1.71	1.61	46.00	28.38	1.30	2.49	0.55
Average (74 samples)...				12.34	1.74	1.63	45.87	28.04	1.24	2.51	0.60
Maximum............				12.91	1.85	1.71	48.06	29.54	1.30	2.58	0.67
Minimum............				11.69	1.64	1.53	43.44	26.10	1.14	2.40	0.53

TABLE 2
Composition of commercially broken-out whole eggs
(Results expressed in percentage)

SAMPLE NO.	SOLIDS	FAT	P₂O₅	TOTAL N.	WATER-SOLUBLE N.
C-1-W	27.52	12.40	0.55	2.16	1.23
C-2-W	26.65	12.07	0.55	2.08	1.19
C-3-W	26.74	12.07	0.61	2.10	1.17
C-4-W	27.29	12.55	0.59	2.11	1.16
C-5-W	26.42	11.79	0.53	2.18	1.20
S-1-W	26.06	11.74	0.52	2.09	1.21
C-6-W	26.75	12.32	0.57	2.08	1.12
C-7-W	26.62	12.29	0.53	2.07	1.16
C-8-W	26.92	12.31	0.57	2.09	1.16
C-9-W	26.98	12.55	0.55	2.06	1.13
S-3-W	25.97	11.58	0.53	2.08	1.18
C-10-W	26.68	11.85	0.54	2.13	1.22
C-11-W	27.23	12.31	0.57	2.12	1.19
C-12-W	26.72	12.20	0.57	2.10	1.13
C-13-W	26.96	12.29	0.55	2.12	1.18
S-5-W	26.82	12.11	0.57	2.07	1.11
S-6-W	27.25	12.53	0.58	2.06	1.13
C-14-W	26.52	12.25	0.59	2.03	1.10
C-15-W	27.35	12.46	0.60	2.12	1.16
C-16-W	26.21	12.06	0.57	2.03	1.08
C-17-W	27.08	12.51	0.56	2.10	1.15
S-7-W	26.62	12.00	0.55	2.07	1.15
S-8-W	27.44	12.44	0.57	2.11	1.20
S-9-W	26.03	11.30	0.51	2.06	1.20
C-18-W	27.17	12.24	0.59	2.14	1.19
C-19-W	27.00	12.37	0.57	2.11	1.18
S-10-W	26.32	11.78	0.53	2.05	1.19
C-20-W	26.86	12.50	0.56	2.03	1.13
C-21-W	27.17	12.21	0.56	2.14	1.19
S-11-W	26.72	11.92	0.55	2.06	1.18
S-12-W	26.41	11.84	0.55	2.06	1.14
S-13-W	26.47	11.84	0.55	2.00	1.15
S-14-W	26.89	11.66	0.55	2.10	1.21
C-22-W	26.85	12.18	0.63	2.09	1.15
C-23-W	27.24	12.36	0.58	2.14	1.19
C-24-W	26.77	12.36	0.55	2.08	1.17
C-25-W	26.29	11.80	0.55	2.07	1.19
S-15-W	26.39	11.89	0.54	2.04	1.16
S-16-W	26.72	12.07	0.55	2.06	1.14
S-17-W	26.66	11.90	0.55	2.07	1.14
S-18-W	26.76	12.00	0.54	2.07	1.17
S-19-W	26.03	11.74	0.54	2.04	1.15
S-20-W	26.54	11.94	0.55	2.08	1.18
C-26-W	26.44	12.06	0.56	2.06	1.14
C-27-W	26.40	11.82	0.57	2.11	1.16

TABLE 2 (*Continued*)

SAMPLE NO.	SOLIDS	FAT	P₂O₅	TOTAL N.	WATER-SOLUBLE N.
S-21-W	26.48	11.79	0.54	2.08	1.14
S-22-W	27.30	12.56	0.58	2.08	1.16
C-28-W	26.34	11.81	0.56	2.05	1.12
C-29-W	26.62	11.99	0.56	2.06	1.16
S-23-W	27.33	12.46	0.58	2.11	1.17
S-24-W	27.02	12.39	0.58	2.07	1.15
C-30-W	26.74	12.08	0.56	2.09	1.17
S-25-W	26.13	11.67	0.53	2.04	1.15
S-26-W	26.42	11.85	0.55	2.06	1.17
C-31-W	27.70	13.03	0.60	2.07	1.12
C-32-W	27.26	12.50	0.56	2.09	1.14
S-27-W	26.93	12.06	0.55	2.10	1.20
S-28-W	26.40	11.58	0.54	2.08	1.17
S-29-W	26.94	11.82	0.55	2.08	1.15
S-30-W	27.33	12.50	0.58	2.08	1.15
C-33-W	26.67	11.92	0.60	2.11	1.17
C-34-W	27.26	12.45	0.60	2.11	1.17
S-31-W	26.97	12.03	0.56	2.07	1.20
S-32-W	26.92	12.17	0.56	2.09	1.20
C-35-W	26.77	12.19	0.59	2.08	1.16
C-36-W	27.30	12.35	0.60	2.14	1.17
C-37-W	26.90	12.50	0.56	2.05	1.14
C-38-W	27.08	12.61	0.56	2.04	1.13
C-39-W	26.52	11.94	0.55	2.10	1.17
C-40-W	26.22	12.02	0.55	2.01	1.11
C-41-W	26.71	12.21	0.58	2.07	1.17
C-42-W	28.33	13.57	0.63	2.11	1.12
C-43-W	26.51	12.09	0.55	2.02	1.15
C-44-W	26.91	12.48	0.58	1.99	1.12
Average (74).	26.80	12.15	0.56	2.08	1.16
Maximum...	28.33	13.57	0.63	2.18	1.23
Minimum...	25.97	11.30	0.51	1.99	1.08

TABLE 3

Composition of commercially separated yolks and whites—dry basis
(Results expressed in percentage)

SAMPLE NO.	WHITES		YOLKS			
	TOTAL N.	WATER-SOL. N	FAT	P₂O₅	TOTAL N.	WATER-SOL. N.
C-1	14.25	13.35	60.79	2.61	5.48	1.37
C-2	14.16	13.43	60.89	2.63	5.40	1.36
C-3	14.43	13.28	61.07	2.71	5.49	1.30
C-4	14.31	13.20	60.45	2.63	5.53	1.37
C-5	14.33	13.14	62.41	2.68	5.39	1.20
S-1	14.4	13.2	61.7	2.72	5.48	1.21

TABLE 3 (*Continued*)

SAMPLE NO.	WHITES		YOLKS			
	TOTAL N.	WATER-SOL. N.	FAT	P₂O₅	TOTAL N.	WATER-SOL. N.
C-6	14.30	13.15	61.66	2.70	5.37	1.20
C-7	14.00	13.17	61.79	2.70	5.46	1.25
C-8	14.24	13.43	61.84	2.72	5.45	1.25
C-9	14.07	13.42	61.10	2.65	5.67	1.38
S-3	14.2	13.5	61.9	2.69	5.43	1.24
C-10	14.26	13.39	60.53	2.67	5.58	1.42
C-11	14.16	13.38	60.36	2.62	5.57	1.43
C-12	14.59	13.62	61.41	2.73	5.49	1.36
C-13	14.44	13.39	61.52	2.81	5.49	1.33
S-5	14.0	13.2	61.1	2.71	5.34	1.21
S-6	13.9	13.1	60.5	2.70	5.46	1.32
C-14	14.34	13.54	60.61	2.67	5.67	1.34
C-15	14.52	13.47	61.16	2.70	5.51	1.34
C-16	14.25	13.43	60.30	2.61	5.75	1.51
C-17	14.31	13.33	61.86	2.70	5.53	1.36
S-7	13.8	12.9	61.3	2.73	5.45	1.25
S-8	13.8	12.9	60.7	2.75	5.46	1.32
S-9	14.0	13.2	61.5	2.76	5.45	1.27
C-18	14.08	13.29	61.06	2.76	5.45	1.39
C-19	14.08	13.20	62.04	2.72	5.35	1.27
S-10	14.0	13.3	61.7	2.72	5.37	1.20
C-20	14.20	13.31	61.47	2.74	5.48	1.36
C-21	14.18	13.13	60.30	2.72	5.59	1.37
S-11	14.0	13.2	60.2	2.73	5.46	1.32
S-12	14.0	13.2	61.3	2.68	5.33	1.17
S-13	13.6	12.7	61.6	2.70	5.20	1.11
S-14	13.7	13.0	61.6	2.77	5.42	1.22
C-22	14.24	13.09	60.44	2.77	5.56	1.34
C-23	14.19	13.22	61.25	2.79	5.45	1.26
C-24	14.14	13.31	61.51	2.71	5.48	1.32
C-25	14.23	13.41	60.93	2.63	5.58	1.35
S-15	13.9	13.0	61.6	2.72	5.33	1.17
S-16	13.9	13.1	61.9	2.72	5.31	1.17
S-17	13.9	13.0	60.9	2.75	5.41	1.28
S-18	14.0	13.0	59.9	2.72	5.59	1.42
S-19	14.3	13.3	61.0	2.74	5.45	1.27
S-20	14.3	13.3	61.7	2.72	5.40	1.17
C-26	14.44	13.22	61.18	2.64	5.49	1.36
C-27	14.34	13.36	61.60	2.71	5.46	1.27
S-21	14.3	13.3	60.9	2.75	5.42	1.25
S-22	14.2	13.2	61.0	2.74	5.46	1.32
C-28	14.13	13.39	61.38	2.79	5.47	1.29
C-29	14.18	13.13	61.47	2.74	5.42	1.19
S-23	14.2	13.3	61.0	2.71	5.34	1.25
S-24	14.1	13.2	60.6	2.74	5.61	1.53
C-30	14.12	13.21	60.90	2.70	5.44	1.30
S-25	14.2	13.3	61.3	2.70	5.35	1.23
S-26	14.1	13.3	60.5	2.72	5.48	1.38

TABLE 3 (*Continued*)

SAMPLE NO.	WHITES		YOLKS			
	TOTAL N.	WATER-SOL. N.	FAT	P₂O₅	TOTAL N.	WATER-SOL. N.
C-31	13.38	12.82	61.54	2.73	5.39	1.24
C-32	14.33	13.25	62.38	2.67	5.38	1.20
S-27	14.2	13.3	61.0	2.73	5.45	1.28
S-28	14.2	13.3	59.9	2.70	5.70	1.51
S-29	14.2	13.3	60.2	2.72	5.49	1.35
S-30	14.0	13.2	60.4	2.73	5.54	1.34
C-33	13.92	13.10	59.94	2.78	5.72	1.49
C-34	13.85	13.12	61.73	2.78	5.43	1.21
S-31	14.3	13.3	61.8	2.75	5.35	1.23
S-32	14.1	13.3	60.6	2.70	5.52	1.43
C-35	14.01	13.36	61.23	2.71	5.47	1.21
C-36	13.96	13.16	62.04	2.74	5.32	1.20
C-37	14.08	13.27	60.93	2.67	5.52	1.43
C-38	14.18	13.07	61.13	2.74	5.51	1.23
C-39	14.15	13.35	61.20	2.66	5.52	1.29
C-40	14.25	13.34	59.85	2.66	5.64	1.49
C-41	13.77	13.27	60.42	2.69	5.52	1.52
C-42	14.03	13.31	61.33	2.67	5.49	1.25
C-43	14.06	12.97	61.54	2.76	5.43	1.35
C-44	13.78	12.97	61.70	2.83	5.41	1.20
Average (74)	14.12	13.23	61.13	2.71	5.47	1.30
Maximum	14.59	13.62	62.41	2.83	5.75	1.53
Minimum	13.38	12.70	59.85	2.61	5.20	1.11

TABLE 4

Composition of commercially broken out whole eggs—dry basis
(Results expressed in percentage)

SAMPLE NO.	FAT	P₂O₅	TOTAL N.	WATER-SOL N
C-1-W	45.06	2.00	7.85	4.47
C-2-W	45.29	2.06	7.80	4.47
C-3-W	45.14	2.28	7.85	4.38
C-4-W	45.99	2.16	7.73	4.25
C-5-W	44.63	2.01	8.25	4.54
S-1-W	45.0	1.99	8.02	4.64
C-6-W	46.06	2.13	7.78	4.19
C-7-W	46.17	1.99	7.78	4.36
C-8-W	45.73	2.12	7.76	4 31
C-9-W	46.52	2.04	7.64	4 19
S-3-W	44.6	2.04	8.01	4.54
C-10-W	44.42	2.02	7.98	4.57
C-11-W	45.21	2.09	7.79	4.37
C-12-W	45.66	2.13	7.86	4.23
C-13-W	45.58	2.04	7.86	4.38

TABLE 4 (*Continued*)

SAMPLE NO.	FAT	P₂O₅	TOTAL N.	WATER-SOL. N
S-5-W	45.2	2.13	7.72	4.14
S-6-W	46.0	2.13	7.56	4.15
C-14-W	46.19	2.22	7.65	4.15
C-15-W	45.56	2.19	7.75	4.24
C-16-W	46.01	2.17	7.75	4.12
C-17-W	46.20	2.07	7.75	4.25
S-7-W	45.1	2.07	7.78	4.32
S-8-W	45.3	2.08	7.69	4.37
S-9-W	43.4	1.96	7.91	4.61
C-18-W	45.05	2.17	7.88	4.38
C-19-W	45.81	2.11	7.81	4.37
S-10-W	44.8	2.01	7.79	4.52
C-20-W	46.54	2.08	7.56	4.21
C-21-W	44.94	2.06	7.88	4.38
S-11-W	44.6	2.06	7.71	4.42
S-12-W	44.8	2.08	7.80	4.32
S-13-W	44.7	2.08	7.56	4.34
S-14-W	43.4	2.05	7.81	4.50
C-22-W	45.36	2.35	7.78	4.28
C-23-W	45.37	2.13	7.86	4.37
C-24-W	46.17	2.05	7.77	4.37
C-25-W	44.88	2.09	7.87	4.53
S-15-W	45.1	2.05	7.73	4.40
S-16-W	45.2	2.06	7.71	4.27
S-17-W	44.6	2.06	7.76	4.28
S-18-W	44.8	2.02	7.74	4.37
S-19-W	45.1	2.07	7.84	4.42
S-20-W	45.0	2.07	7.84	4.45
C-26-W	45.61	2.12	7.79	4.31
C-27-W	44.77	2.16	7.99	4.39
S-21-W	44.5	2.04	7.85	4.31
S-22-W	46.0	2.12	7.62	4.25
C-28-W	44.84	2.13	7.78	4.25
C-29-W	45.04	2.10	7.74	4.36
S-23-W	45.6	2.12	7.72	4.28
S-24-W	45.9	2.15	7.66	4.26
C-30-W	45.18	2.09	7.82	4.38
S-25-W	44.7	2.03	7.81	4.40
S-26-W	44.9	2.08	7.80	4.43
C-31-W	47.04	2.17	7.47	4.04
C-32-W	45.85	2.05	7.67	4.18
S-27-W	44.8	2.04	7.80	4.46
S-28-W	43.9	2.05	7.88	4.43
S-29-W	43.9	2.04	7.72	4.23
S-30-W	45.7	2.12	7.61	4.21
C-33-W	44.69	2.25	7.91	4.39
C-34-W	45.67	2.20	7.74	4.29
S-31-W	44.6	2.08	7.68	4.45
S-32-W	45.2	2.08	7.76	4.46

SAMPLE NO.	FAT	P_2O_5	TOTAL N.	WATER-SOL N
C-35-W	45.54	2.20	7.77	4.33
C-36-W	45.24	2.20	7.84	4.29
C-37-W	46.47	2.08	7.62	4.24
C-38-W	46.56	2.07	7.53	4.17
C-39-W	45.02	2.07	7.92	4.41
C-40-W	45.84	2.10	7.67	4.23
C-41-W	45.71	2.17	7.75	4.38
C-42-W	47.90	2.22	7.45	3.95
C-43-W	45.60	2.07	7.62	4.34
C-44-W	46.38	2.16	7.39	4.16
Average (74).....	45.34	2.10	7.76	4.33
Maximum.......	47.90	2.35	8.25	4.64
Minimum.......	43.40	1.96	7.39	3.95

MICRO ANALYTICAL METHODS[1]

By E. P. CLARK (Insecticide Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture)

The modern tendency in organic chemical analysis is to adopt methods involving operations upon relatively small quantities of material. This practice is the direct outcome of the work upon many recent problems in organic chemistry concerned with materials available only in minute quantities, the study of which demanded analytical procedures employing correspondingly small samples. Efforts in devising such methods have resulted in remarkable procedures; in fact they have developed an important new branch of chemistry.

The outstanding accomplishment in this field is Pregl's system of quantitative organic microanalysis[2] in which samples of from 2 to 5 mg. are generally taken for analysis. This system consists of well-developed methods that are as accurate as the standard methods for the determination of various elements, groups, and constants. Aside from economy of material, which was primarily responsible for its development, Pregl's system of analysis also requires less time, reagents, and space for the execution of the work. These factors are generally considered so important that even when a plentiful supply of material to be analyzed is available, the system is preferred. Nor is this general adoption confined to research chemists; more and more analysts in routine and control laboratories have been applying its principles to their activities. It is undoubtedly true that an appreciable saving in the financial upkeep of the laboratories using this

[1] Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., November, 1932.
[2] Quantitative Organic Microanalysis. Translated by Fyleman. Blakiston's Sons and Co., Philadelphia (1930).

system has been realized. Gesell and Dittmar,[1] reporting in 1925 from the laboratories of Lehn and Fink, Inc., stated that in control laboratories of large concerns where costs run from $20,000 to $100,000 a year a financial saving of from 50 to 60 per cent is indicated when micro methods are adopted.

Notwithstanding these considerations, however, the change from macro to micro methods in routine and control work has not had the response that appears warranted. The reason for this conservatism is perhaps due to the fact that with all its advantages the Pregl system has two characteristics that cause some analysts to hesitate in adopting it. It is recognized that the Pregl system demands a higher degree of skill on the part of the analyst than is required in the use of the standard macro system and that the technic involves many seemingly trivial details that must be strictly followed. If continued over long periods, work of this type is tedious, and many chemists lack the temperament for its execution. A more important consideration perhaps is that for the most part the work must be done in a room in which no other activity is conducted, and frequently the arrangement of a laboratory or the particular conditions existing are not suitable.

These considerations have from time to time given rise to the idea in the minds of many chemists that, except for the type of work in which material is at a great premium, a system employing somewhat larger analytical samples than are worked with in the Pregl plan would be preferable. Such a system should possess the economical advantages of the micro plan and still enable the analyst to conduct his work in a manner and under conditions comparable to those used in the standard macro system. With these characteristics it should make a special appeal to chemists concerned mainly with analytical or control work.

This idea has been given careful consideration, and such a system has been utilized by the writer for the past four years with most gratifying results. Applied to the work for which it was designed it proved to be not only extremely desirable but for some determinations the procedures are believed to be superior to any previously employed.

In the plan which was adopted analytical samples ranging from 5 to 25 mg. were usually employed. It was found, as predicted from mathematical and other considerations, that operations upon quantities of this magnitude compared favorably in economy of time, space, and reagents with the Pregl system, and in addition had the distinct advantage that requirements as to details, laboratory conditions, balances, etc., are not nearly so exacting as those imposed by the micro system.

Also with the wealth of fundamental information available as a result of the researches of Pregl and his students, it was not difficult to develop smoothly-working semi micro methods of this type for most of the deter-

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[1] *Ind. Eng. Chem.*, 17, 808 (1925).

minations required. Because of the excellent results obtained by this system of analysis; the advantages it possesses from the standpoint of workability and economy of time, reagents, and space; and also because of the writer's confidence that the system could be advantageously applied to the type of work in which this Association is particularly interested, a brief outline of its development as made by the writer is submitted for consideration.

The work that gave rise to this plan of analysis was concerned for the most part with the study of the structures of relatively complex organic compounds. The materials, while available in moderate quantities, were not sufficiently plentiful to allow the use of the standard macro methods of analysis. This was especially true of many of the derivatives of the mother substances studied. Consequently, in these investigations the determinations of carbon, hydrogen, nitrogen, halogens, the methoxyl and carboxyl groups, and molecular weights were most frequently required.
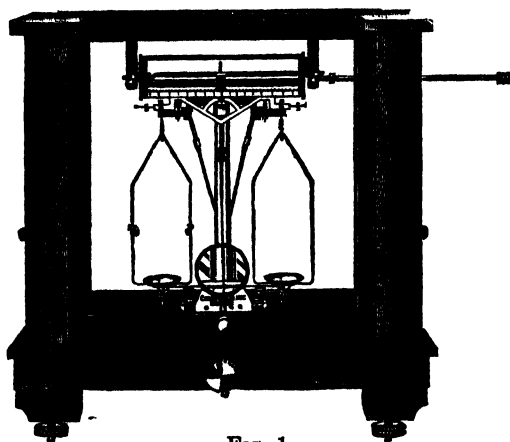
FIG. 1

The Balance.—Accurate analytical work in which quantities of from 5 to 25 mg. are employed necessitates a balance of greater delicacy than that used in macro analysis. No satisfactory balance of the type desired was available at the time this work was undertaken, but through the assistance and cooperation of Christian Becker, Inc., which at this time is gratefully acknowledged, an excellent instrument sensitive to 0.01 mg. was built[1] (Fig. 1). The balance is responsive to an unusual degree; it keeps its zero point exceedingly well, and its action is more rapid than a first-class analytical balance with a sensitivity of 0.1 mg. An important feature of the instrument is that, with simple precautions, weighings can be made with it as rapidly and freely as with any good analytical balance.

[1] This balance has since been placed upon the market by Christian Becker, Inc., 92 Reade St., New York, N. Y.

An outline of some of the determinations most frequently required in the work referred to will be presented to indicate how the system operates.

Carbon and Hydrogen.—Carbon and hydrogen determinations were usually made upon 20–25 mg. of material. An electrically heated combustion furnace was built to take a tube 11 mm. inside diameter and 60 cm. long. Pregl's universal tube filling, supplemented by a cerium dioxide catalyst, was employed, and ascarite and calcium chloride tubes, similar to Pregl's but larger (approximately 100×14 mm.), were used to absorb CO_2 and water. The combustion was conducted in essentially the same manner as in a macro determination. The Mariotte flask required by the micro method was found to be unnecessary, and so far all compounds have been successfully burned in pure oxygen. The absorption tubes containing pure oxygen were weighed before and after the combustion so that some time was saved in sweeping the system free from oxygen as is done in the usual procedures. The absorption tubes were not weighed upon the micro balance but upon a balance sensitive to 0.1 mg. The time required to make a determination varied somewhat with the nature of the compound, but frequently as many as eight combustions have been made in a working day.

Nitrogen.—Nitrogen was determined by both the Dumas and Kjeldahl methods. In the Dumas method samples weighing from 15 to 25 mg. were employed, depending upon the quantity of nitrogen in the compounds. The procedure was essentially the same as that used in a macro determination, except that the size of the equipment was considerably reduced. The graduated portion of the azotometer was made of tubing 8 mm. outside diameter and 30 cm. long. It was graduated in 0.05 cc. units so that the volume could easily be read to 0.01 cc. The graduated portion of the azotometer was sealed to a tube 40 cm. long with an outside diameter of 2 cm. This tube held most of the potassium hydroxide solution. The sample was placed with copper oxide powder in a relatively long boat made of thin copper foil. Carbon dioxide was generated from a saturated solution of potassium bicarbonate. The results obtained were always of a high order of accuracy.

For Kjeldahl determinations, 5–10 mg. samples were usually taken. The procedure is the same as that recommended by Pregl except that an electrically operated Parnass-Wagner apparatus[1] was used for the distillations. By making the distillation apparatus somewhat larger than usual (Figs. 2 and 3), and employing N/30 acid and alkali, 20-mg. samples weighed upon an ordinary analytical balance may be run by the usual Pregl method with remarkably accurate results. This procedure was successfully used by the writer for several years before facilities were available for the micro system.

[1] Clark and Collip, *J. Biol. Chem.*, **67**, 621 (1926).

Halogens.—The determination of halogens was carried out upon samples of 25 mg., mostly by the Carius method. The procedure is the same as that of the macro method, except that the tubes employed are 11 mm.

FIG. 2.—MODIFIED PARMAS-WAGNER MICRO-KJELDAHL
DISTILLING APPARATUS

FIG. 3.—MICRO-KJELDAHL DIGESTER

outside diameter by 18 cm. long. It has been the practice to collect the silver halide upon an asbestos mat in Jena sintered glass filtering tubes No. 154 G 1. The procedure for transferring the precipitate is that recommended by Pregl.

Many methoxyl determinations employing 20 mg. samples were made by the Zeisel and the Vieböck and Schwappach methods, but since these methods have already been described in detail[1] no further discussion of them will be presented.

This brief description gives a general outline of what has been accomplished. It should be borne in mind that these analytical procedures were a means to an end, and no particular study of the semi-micro system as such was made other than that necessary to meet the immediate requirements of the problems at hand. From the experience gained, however, the writer ventures the suggestion that such an analytical system has many possibilities for the type of work in which this Association is interested.

OCCURRENCE OF SULFUR, ORGANIC MATTER, NITROGEN, AND WATER IN PHOSPHATE ROCK

By W. L. HILL, H. L. MARSHALL, and K. D. JACOB (Fertilizer and Fixed Nitrogen Investigations, Bureau of Chemistry and Soils, Washington, D. C.)

An extensive investigation on the composition of phosphate rock has been under way in this Bureau for several years, and the results on certain phases of the subject have been reported[2] from time to time. In this work rather complete chemical analyses were made on about sixty samples of material from various deposits in the United States and elsewhere. In connection with complete analyses it is desirable to have quantitative data on the condition of the sulfur and on the amount, as well as the composition, of the organic matter occurring in the material. Therefore detailed results were obtained on the several forms of sulfur occurring in domestic rock phosphate, and total hydrogen, organic carbon, and nitrogen were also determined.

A description of the materials used was given in the papers cited above, and therefore it is sufficient to state that the samples were representative of commercial material which is now, or has been, produced in the particular localities. Furthermore, it should be pointed out that the phosphate rock of commerce, in the course of its preparation for the market, is subjected to an artificial drying process which may affect the results for organic carbon. As indicated in Table 8, a number of the samples used in this study were taken from materials which had not been dried artificially.

[1] Clark, *J. Am. Chem. Soc.*, **51**, 1479 (1929); *This Journal*, **15**, 136 (1932).
[2] *This Journal*, **11**, 237 (1928); *Colloid Symposium Annual*, **7**, 195 (1930); *Ind. Eng. Chem.*, **21**, 1253 (1929), **22**, 1392 (1930), **23**, 1120 and 1413 (1931), **24**, 86, and 1306 (1932); *This Journal*. **16**, 128 (1933).

In most instances the results are the mean of duplicate determinations, and all results were calculated to a moisture-free basis (105° C.).

ANALYTICAL METHODS
I. SULFUR

Total sulfur.—Total sulfur was determined by fusion with sodium carbonate and potassium nitrate according to the procedure described by Hillebrand.[1]

Fractional Determinations.—The forms of sulfur usually determined in rocks, viz., acid-soluble sulfide and sulfate and acid-insoluble sulfide and sulfate, were determined on the same sample in accordance with the general method briefly outlined by Hillebrand.[2] The details of the procedures actually employed are described briefly in the following paragraphs.

The acid-soluble sulfide was volatilized as H_2S by boiling 2 grams of the sample with 100 ml. of hydrochloric acid (1+4) in an atmosphere of nitrogen in a closed system,[3] and the evolved hydrogen sulfide swept into an absorption bulb containing ammoniacal cadmium sulfate solution. The absorbent was diluted, acidified with hydrochloric acid, and titrated with 0.03 N iodine solution, starch being used as an indicator.

If a qualitative test with lead acetate paper indicated the absence or the presence of only a trace of H_2S when the sample was treated with acid, the initial acid treatment conducted in an atmosphere of carbon dioxide in a covered 250 cc. beaker.

The cooled contents of the reaction flask, or beaker, were filtered through a thin mat of asbestos in a special filtration apparatus,[4] and the insoluble residue was washed with cold hydrochloric acid (1+20). The usual precautions for preventing oxidation of pyritic sulfur were observed during the filtration and washing. After the filtrate had been diluted to a volume of 450 ml. and the acidity adjusted to about 1 ml. of concentrated hydrochloric acid per 100 ml. of solution, the acid-soluble sulfate was precipitated at the temperature of the steam bath with 3 per cent barium chloride solution.

The acid-insoluble sulfide was determined on the residue from the initial acid treatment. Thus, the residue was treated, according to the method of Smoot,[5] with a mixture of nitric and hydrochloric acids containing a few drops of bromine. After the oxidation treatment and the removal of nitrates, the residue was digested with 1 ml. of concentrated hydrochloric acid and 25 ml. of hot water, filtered, and washed. On washing with water, turbid filtrates were often obtained. In some instances the turbidity was due to organic matter which had escaped oxidation, and which even re-

[1] U. S. Geol. Survey Bull., **700**, 170, 230 (1919).
[2] *Ibid.*, 233.
[3] Scott, Standard Methods of Chemical Analysis, Vol. I, p. 501, Van Nostrand (1922).
[4] Lord and Demorest, Metallurgical Analysis, p. 123, McGraw-Hill (1916)
[5] Hillebrand and Lundell, Applied Inorganic Analysis, p. 572, Wiley (1929).

sisted two successive oxidation treatments; in other cases it was evidently caused by finely-divided silica. However, this difficulty can be prevented by using dilute hydrochloric acid as a wash liquid. The sulfate in the clear filtrate and washings was determined by precipitation with barium chloride.

The insoluble sulfate was determined on the residue from the decomposition of insoluble sulfides by the method used in the analysis for total sulfur, potassium nitrate being omitted from the fusion mixture. In a few cases sulfur was determined in the washed residue, after the rock had been treated with hydrochloric acid (1+4), by carefully igniting the moist filter under a layer of a mixture of magnesium oxide and sodium carbonate according to Eschka's method[1] for the determination of sulfur in coal.

II. ORGANIC MATTER

Organic carbon and water.—The procedure described by Hillebrand and Lundell[2] for determining organic carbon and hydrogen in rocks by direct combustion was employed, care being taken to observe the precautions required in the presence of halogens, sulfur, and nitrogen. Accordingly, 1–3 grams of the sample (100-mesh), previously dried to constant weight at 105°C., was burned directly in oxygen in a quartz tube at a temperature of 800°C., and the carbon dioxide by combustion was corrected for the carbon dioxide evolved from carbonates originally present in the material. The magnitude of this correction, that is the difference between the amounts of carbon dioxide found in the original material and the burned sample by evolution with boiling dilute hydrochloric acid, varied from 0.05 to 4.83 per cent, and depended to a considerable extent upon the type of phosphate. If phosphate rock, like soils,[3] contains organic compounds that yield carbon dioxide when boiled with acid, the carbonate correction found in this manner is somewhat high, and as a consequence the results for organic carbon are low. However, in view of the short period of boiling used in the determination of carbon dioxide and the fact that in several tests aspiration with cold dilute acid, as suggested by Alexander and Byers,[4] gave almost exactly the same results as did the treatment with boiling acid, the error arising from this source is regarded as negligible.

With phosphate rock containing appreciable amounts of pyritic sulfur, a finely-powdered mixture of lead chromate and red lead oxide (4+1) was added to the dried sample immediately before it was introduced into the combustion tube. In this case the combustion was made at 600° C., and dilute phosphoric acid was used in determining the residual carbon dioxide. Results for carbon and water determined on *pyrite-free* phosphates

[1] Scott, Standard Methods of Chemical Analysis, Vol. I, p. 495. Van Nostrand (1922).
[2] *Loc. cit.*, 625–30.
[3] Shorey and Martin, *J. Am. Chem. Soc.*, 52, 4907 (1930).
[4] U. S. Dept. Agr. Tech. Bull., 317 (1932).

in this manner were in good agreement with the figures obtained at 800° C. without the addition of lead chromate.

The accuracy of the figures for organic carbon, as determined by direct combustion of the sample, is impaired more or less by the correction for carbonate carbon. The necessity for this correction is avoided in the method described by Fieldner, Selvig and Taylor[1] for determining combustible matter in silicate and carbonate rocks, according to which carbon is determined in the dry organic matter actually isolated from the sample by an acid treatment. In this method, however, any soluble organic material is lost in the filtrates, and besides this, losses may be incurred as a result of hydrolytic and oxidation processes during the digestion with acid and subsequent filtration and washing. For purposes of comparison organic carbon was determined in several typical samples of phosphate rock by this method, the procedure recommended for carbonate rocks being used. The results (Table 1) indicate considerable losses of carbon in

TABLE 1

Comparison of results for organic carbon by combustion methods.

SAMPLE NUMBER	TYPE OR SOURCE OF PHOSPHATE	HYDROGEN FROM ISOLATED ORGANIC MATERIAL, AS H_2O	ORGANIC CARBON	
			BY DIRECT COMBUSTION OF ROCK	BY ISOLATION AND COMBUSTION
		per cent	*per cent*	*per cent*
910[a]	Florida land pebble	0.14	0.33	0.18
912[a]	Florida land pebble	0.12	0.38	0.11
771	Florida hard rock	0.16	0.52	0.24
932[a]	Florida hard rock	0.04	0.18	0.06
906[a]	Tennessee brown rock	0.04	0.09	0.11
908[a]	Tennessee brown rock	0.07	0.11	0.14
930[a]	Tennessee blue rock	0.16	0.20	0.28
454	Idaho	0.85	2.29	2 33
973	Idaho	0.77	2.35	2.22
552	Tunis	0.52	0.86	0.73
985	Curaçao Island	0.02	0.17	0.06

[a] Air-dried material.

Florida land pebble, Florida hard rock and Curaçao phosphate as a result of the isolation treatment. On the other hand, the results by the two methods are in good agreement on Tennessee brown rock and blue rock, Idaho rock, and Tunis phosphate.

Colorimetric Method for the Determination of Organic Matter.—C. A. Butt of the International Agricultural Corporation, East Point, Ga., developed a rapid control method for estimating the organic matter in Florida phosphates. His procedure[2] is as follows:

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[1] Bur. Mines Tech. Paper, 212 (1919).
[2] Private communication.

Weigh 1 gram of the finely-ground sample into a suitable beaker, add 50 cc. of C. P. sulfuric acid, sp. gr. 1.84, and heat to fuming on the hot plate. Cool, and compare the depth of reddish color with standards to arrive at the percentage of organic matter.

Make up the standards as follows: Ignite a sample of rock, similar in composition to the sample to be analyzed, at bright red heat. To 1 gram portions of this ignited rock, add dry powdered sugar in amounts equivalent to 0.20, 0.40, 0.60, 0.80 per cent of the rock, and treat as above.

It was suggested that this method is best suited to Florida phosphates with a low iron content. In the preparation of standards, P. McG. Shuey of Savannah, Ga., recommends[1] tannic acid, because it yields a coloration which is more easily compared with that given by the organic matter in the rock.

Although this method cannot be expected to yield accurate results, it may give figures which indicate the relative amounts of organic matter in various samples of a given type of rock. In view of the fact that complete destruction of the carbonaceous matter in phosphate rock is not always readily effected by ignition, the ignited rock should be tested for residual organic material capable of imparting color to the acid solution.

The results obtained in this manner on Florida land-pebble phosphate are given in Table 2. For comparison, results for organic matter (6th column) were calculated from the organic carbon content of the materials,

TABLE 2

Organic matter in Florida land-pebble phosphate as determined by the colorimetric method.

SAMPLE NUMBER	TOTAL IRON, AS Fe_2O_3	ORGANIC MATTER BY THE COLORIMETRIC METHOD		ORGANIC CARBON BY DIRECT COMBUSTION	ORGANIC MATTER CALCULATED FROM ORGANIC CARBON
		IN TERMS OF SUGAR	IN TERMS OF TANNIC ACID[a]		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
912	0.70	1.6	0.90	0.38	0.72
619	1.63	2.0	1.03	0.33	0.57[b]
947	1.69	1.6	0.95	0.29	0.50[b]
439	1.90	1.2	0.70	0.25	0.43[b]
910	2.59	1.6	0.95	0.33	0.52

[a] Average of two separate determinations, the maximum deviation of the results being 0.1 per cent.
[b] Result calculated with the aid of the mean of the percentages of carbon in the organic material isolated from Nos. 910 and 912 (Table 9).

as determined by direct combustion of the rock sample, and the percentage of carbon found in the isolated organic matter (Table 9). The accuracy of these calculated values is probably not high as considerable carbon was lost during the isolation treatment (Table 1), but in view of the tendency of carbon to concentrate in organic residues, results obtained in this manner are most likely lower than the true values. The

[1] Private communication.

range of the calculated results is considerably lower than that of the colorimetric results. The figures for organic matter in terms of sugar are unquestionably much too high; and although those obtained with tannic acid are also most likely high, they are, in comparison with the calculated values, fairly close to the percentages that may be expected. Thus, the colorimetric results with tannic acid, which are roughly proportional to the figures for organic carbon, and the calculated values may be regarded as the upper and lower limits, respectively, between which lie the true percentages of organic matter in these materials. Finally, it may be stated that the results obtained by the colorimetric method on Tennessee brown rock, Tennessee blue rock, and Idaho phosphate were entirely unsatisfactory.

Organic Matter and Water from the Ignition Loss.—In technical analyses of phosphate rock a figure for "organic matter and combined water" is frequently calculated from the total ignition loss by deducting from the latter the percentage of carbon dioxide in the sample. In view of this custom it is desirable to compare the results by this method with those obtained by the direct determination of carbon and water. For this purpose the ignition loss was determined by the following method:

> Weigh 2–3 grams of the 100-mesh sample into a platinum dish of such size that the depth of the material is 0.5 cm. or less, and dry in an electric oven to constant weight at 105° C. to determine the moisture. Place the dried sample in a central position on the floor of an electric muffle furnace heated to 1000° C., and ignite for one hour with the furnace door opened about 0.75 inch to admit air, keeping the temperature between 990° and 1010° C. as indicated by an electric pyrometer. Cool the ignited sample in a desiccator over concentrated sulfuric acid, weigh, and re-ignite for 30-minute periods until the change in weight between two successive ignitions is not more than 0.5 mg.

The errors involved in this method of determining organic matter and water have been discussed by Hillebrand and Lundell,[1] and it is otherwise well known that the results thus obtained are, in general, only rough approximations. Quantitative data relating to the behavior of certain constituents other than organic matter and water, which cause considerable alterations in weight during the ignition of phosphate rock, are given in Tables 3, 4, and 5. It will be noted that the carbon dioxide (Table 3) is less readily expelled from phosphate rock than from limestone or dolomite, and that appreciable amounts of carbonate carbon are retained by the phosphates after ignition to constant weight at 1000° C. Moreover, temperatures requisite for complete expulsion of carbonate carbon lead to a considerable loss of fluorine (Table 4). Although the fluorine escapes principally in the form of silicon tetrafluoride, it may, and in some cases actually does, escape in other forms. It may be pointed out in this connection that, as indicated by a single analysis, the loss of silicon from the

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[1] *Loc. cit.*, pp. 689–90·

pyrite-bearing Tennessee blue rock was almost negligible. With regard to the change in sulfate content on ignition (Table 3) it may be pointed out that appreciable increases in this form of sulfur occurred in certain pyrite-free phosphates, namely, Florida hard rock, Idaho rock, and Curaçao phosphate. This effect is attributed to the oxidation of organic sulfur. When phosphate rock that contains a considerable amount of pyrite is ignited, at

TABLE 3

Behavior of carbon dioxide and sulfate on ignition

SAMPLE NUMBER	MATERIAL	IGNITION LOSS AT 1000°C.[a]	CO$_2$		ACID-SOLUBLE SULFATE, AS SO$_3$	
			ORIGINAL MATERIAL	IGNITED MATERIAL	ORIGINAL MATERIAL	IGNITED MATERIAL
		per cent	per cent	per cent[b]	per cent	per cent[b]
910	Florida land-pebble phosphate	6.60	3.76	0.33	0.98	0.96
912	Florida land-pebble phosphate	3.10	1.48	0.43	0.20	0.23
932	Florida land-pebble phosphate	3.19	2.19	0.70	0.01	0.29
908	Tennessee brown-rock phosphate	3.22	1.96	0.50	0.70	0.65
772[c]	Tennessee blue-rock phosphate	5.42	2.00	0.00	4.02	3.75
930[c]	Tennessee blue-rock phosphate	4.32	2.59	0.15	1.26	2.87
1049[c,d]	Tennessee blue-rock phosphate	4.90	0.91	0.20	2.00	1.14
1048	Tennessee white-rock phosphate	3.16	2.36	0.93	0.02	0.01
454	Idaho phosphate	6.50[e]	1.80	0.15	1.66	1.81
973	Idaho phosphate	6.49[e]	1.72	0.03	1.69	1.78
1139[e]	South Carolina phosphate	8.69	5.06	0.25	1.74	2.00
1162	Morocco phosphate	5.72	4.12	0.28	1.40	1.45
985	Curaçao phosphate	4.85	3.90	1.89	0.61	0.69
971	Bone ash	0.98	0.70	0.08	0.40	0.44
916[c]	Tennessee phosphatic limestone	28.48	28.22	0.23	1.55	2.45
1224	Dolomite	44.91	—	0.01	—	—
1135	Limestone	43.56	—	0.03	—	—

[a] Ignited to constant weight.
[b] Per cent of original material.
[c] Pyrite-bearing material.
[d] Kidney phosphate.
[e] Ignited at 1050° C.

least a part of the pyritic sulfur is expelled regardless of the furnace conditions (Table 5), whereas the other part is retained in the residue as sulfate; in certain instances all the pyritic sulfur and part of the sulfate is volatilized, for example, Samples 772 and 1049 (Table 3).

In the last column of Table 8 the results for organic matter and water obtained by deducting the carbon dioxide in the sample from the ignition loss are given. It will be noted that, as a rule, the results by direct determination (Table 8, column 7), which include any water arising from the combustion of organic matter, are higher than the values obtained from the ignition loss.

In order to obtain a more reliable result from the ignition loss, some authors analyze the ignited residue for certain constituents that are in part volatilized, and, on the basis of certain assumptions regarding the behavior of these constituents, correct the ignition loss accordingly. For example, Rosonow,[1] working with Russian pyrite-bearing phosphorites,

TABLE 4

Behavior of fluorine on ignition at 1000°C[a]

| SAMPLE NUMBER | TYPE OF SOURCE OF PHOSPHATE | FLUORINE, % OF SAMPLE | |
		ORIGINAL MATERIAL	VOLATILIZED
		per cent	per cent
790	Florida land pebble	3.97	0.50
762	Tennessee brown rock	3.87	0.48
930[b]	Tennessee blue rock	3.80	0.63
948[b]	Wyoming	3.54	0.40

[a] Five gram samples were ignited for 30 minutes. The ignitions and fluorine analyses were conducted by D. S. Reynolds in connection with his work on the volatilisation of fluorine from phosphate rock.
[b] Pyrite-bearing material.

TABLE 5

Effect of temperature on the ignition loss and residual sulfur when pyrite is present

| TEMP. | BUR. STANDARDS STANDARD SAMPLE NO. 56[a] | | TENNESSEE BLUE-ROCK PHOSPHATE NO. 772[b] | |
	IGNITION LOSS	SULFUR IN RESIDUE, AS SO_3[c]	IGNITION LOSS	SULFUR IN RESIDUE, AS SO_3[c]
°C.	per cent	per cent	per cent	per cent
500	1.21	1.50	1.36	4.60
600	1.40	1.65	1.98	4.45
700	1.61	1.52	1.97	4.65
800	1.78	1.66	2.00	4.98
800[d]	2.06	1.73	2.43	4.57
1000	3.77	1.50	4.87	3.75

[a] Pyrite-bearing Tennessee brown-rock phosphate, containing (as SO_3) 1 24% sulfate and 1.19% pyritic sulfur.
[b] Sulfur content, as SO_3: sulfate, 4.02; pyritic sulfur, 2 71%.
[c] Percentage of unignited sample.
[d] Furnace door closed. Other ignitions were made with furnace door opened 0 75 inch.

ignited the sample for 3 hours at 850°C., determined the carbonate carbon and sulfur both in the ignited sample and in the original material, and by means of these data applied a correction to the ignition loss, at the same time considering the fluorine loss to be negligible. Although no corrections of this kind have been applied to the results given in this paper, the authors have taken into account the behavior of pyrite in connection with the totals of complete analyses. Thus, the correction to be added algebraically to the ignition loss is given by the equation, $c = s - (a + p/4)$, in which c

[1] *Z. anal. Chem.*, **83**, 410 (1931).

is the correction, a and p the percentages of sulfate and pyritic sulfur (considered as SO_3), respectively, in the unignited material, and s is the percentage (on the original material) of sulfate sulfur in the pyrite-free ignited sample. In the development of this simple equation it is assumed that the pyritic iron is completely oxidized to Fe_2O_3.

OCCURRENCE OF SULFUR

The results for total sulfur (Table 6) indicate that appreciable amounts of sulfur are present in all domestic rock phosphates, and that, for a given type of phosphate, the materials relatively high in phosphoric anhydride usually contain the smallest percentages of sulfur. Florida hard rock, pyrite-free Tennessee brown rock, and Montana phosphate consistently show less than 1 per cent of sulfur, while Tennessee blue rock is considerably richer in this element than any of the other types of phosphate for which data are available. The percentage of sulfur in the single sample of Florida hard rock included in the table is unusually high for this type of phosphate. Thus, the range of the results on three additional hard-rock samples is 0.12–0.16 per cent, which agrees favorably with the results on four samples as reported by F. W. Clarke.[1]

Previous data relating to the total sulfur content of Florida land-pebble and Tennessee brown-rock phosphates have not come to the authors' attention. A few results for sulfur, presumably total sulfur, in several other types of domestic phosphate are available. Thus, sulfur in amounts ranging from 2 to 3 per cent has been reported in phosphate rock from Idaho and Utah,[2] and Montana;[3] somewhat smaller amounts in phosphates from South Carolina[4] and Johnson County,[5] Tennessee; and traces have been found in North Carolina[6] and Oklahoma[7] phosphates.

A few references to the condition of the sulfur in phosphate rock may be cited. Sulfate sulfur (SO_3) has been reported in Florida land pebble[8] and Tennessee blue rock.[9] Figures for pyritic sulfur in Tennessee blue rock are given in the latter reference and by Hook.[10] Jones[11] found sulfate and iron sulfide in Utah phosphate.

It will be noted (Table 6) that a considerable part of the total sulfur is present as sulfate in all phosphates analyzed except Florida hard rock. Furthermore, the sulfur is usually present in more than one inorganic form. Small quantities of acid-insoluble sulfates frequently occur, particularly in Florida pebble. In this connection, it may be mentioned that

[1] U. S. Geol. Survey Bull., **770,** 533 (1924).
[2] U. S. Geol. Survey Professional Paper, **152,** 210 (1927); *Ind. Eng. Chem.*, 21, 1172 (1929), 22, 242 (1930).
[3] U. S. Geol. Survey Bull., **640,** 221 (1917).
[4] U. S. Geol. Survey Mineral Resources for 1882, 504 (1883); U. S. Geol. Survey Bull., 46, 69 (1888); Millar, Florida, South Carolina, and Canadian Phosphates. London (1892).
[5] *Resources of Tennessee*, **6,** 51 (1916).
[6] U. S. Geol. Survey Mineral Resources for 1883–4, 793 (1885).
[7] Oklahoma Univ. Bull., 271, 97 (1923); *Chemical Age* (New York), 31, No. 7, 319 (1923).
[8] Gray, Phosphates and Superphosphates, p. 30. London (1930).
[9] U. S. Geol. Survey, 16th Ann. Rept., pt. 4, 631 (1895).
[10] *Resources of Tennessee*, **4,** 51 (1914).
[11] *Trans. Am. Inst. Mining Eng.*, **47,** 192 (1914).

barium[1] was also found in these phosphates. Measurable quantities of soluble sulfides were found in Florida pebble, and somewhat larger amounts in Idaho and Wyoming phosphates. This form of sulfur was not found in Florida hard rock, pyrite-free Tennessee brown rock, Montana phosphate, the island phosphates, or the phosphates from North Africa. Relatively large amounts of pyritic sulfur are present in Tennessee blue rock and phosphatic limestone. The range of the results on the former, 1.37–5.21 per cent in terms of SO_3, is considerably higher than previous figures for pyritic sulfur in blue rock. Somewhat smaller percentages of acid-insoluble sulfides were found in Wyoming phosphate, one sample of Tennessee brown rock, and South Carolina phosphate.

The results for the four inorganic forms of sulfur (Table 6) show that all the sulfur is not recovered in the fractional determinations. As indicated in the last column of this table, the differences range from 0.01 per cent on Florida pebble to 0.81 per cent on phosphate from Conda, Idaho, and are too large in many cases to be ascribed to analytical error. Moreover, the greatest differences are noted in material that is rich in organic carbon. In view of this observation and the fact that the organic material separated from several types of phosphate contains relatively large percentages of sulfur (Table 9), it is concluded that the results given in the last column of Table 6, aside from the relatively small analytical errors accumulated therein, represent the amounts of organic sulfur present in these materials. Figures for the acid-insoluble part of the organic sulfur in several typical samples of phosphate rock, as determined by Eschka's method, are given in Table 7, where, for the sake of comparison, the figures given in the last column of Table 6 are also shown. In most cases the result obtained by Eschka's method (2nd column) is, within the limits of analytical error, equal to the difference between the total sulfur and the sum of the fractional determinations, and in these instances the entire sulfur content of the material has been duly accounted for.

OCCURRENCE OF ORGANIC MATTER, NITROGEN, AND WATER

Since nitrogen is a constituent of the organic matter occurring in phosphate rock (Table 9) and since the figures for water include the water arising from the combustion of the organic material, the results for these constituents will be discussed together. The results for organic carbon, which also include elementary carbon, will be considered as a measure of the amounts of organic matter present; and the figures for water, as already indicated, represent the total water, exclusive of moisture expelled at 105° C., given off in a dry oxidizing atmosphere at a temperature of 800° C. (600° C. with pyrite-bearing phosphates).

The occurrence of appreciable quantities of organic matter in domestic phosphate rock is well known. Clarke[2] reported 0.12 to 0.22 per cent of

[1] *Ind. Eng. Chem.*, **24**, 1306 (1932).
[2] U. S. Geol. Survey Bull., **770**, 553 (1924).

TABLE 6

Sulfur content of phosphate rock

SAMPLE NUMBER	SOURCE OF PHOSPHATE	P₂O₅	FRACTIONAL FORMS OF SULFUR, AS SO₃				TOTAL SULFUR, AS SO₃	TOTAL SULFUR MINUS SUM OF FRACTIONAL DETERMINATIONS
			ACID-SOLUBLE SULFATE	ACID-INSOLUBLE SULFATE	H₂S EVOLVED ON TREATMENT WITH ACID	ACID-INSOLUBLE SULFIDE		
		per cent	per cent	per cent	per cent	per cent	per cent	per cent
	Florida Land-Pebble Phosphate							
618	Pierce	30.53	1.14	0.20	0.04	—	1.42	0.04
617	Brewster	30.70	1.19	0.13	0.05	0.02	1.51	0.12
619	Nichols	30.98	1.06	0.07	0.04	—	1.29	0.12
910	Mulberry	31.09	0.98	0.00	0.05	—	1.05	0.02
947	Brewster	31.28	1.09	0.05	0.03	—	1.22	0.05
790	Unknown	31.40	1.19	0.00	0.03	0.00	1.36	0.14
627	Lakeland	33.70	0.85	0.01	0.01	—	1.08	0.21
912	Mulberry	35.37	0.20	0.00	0.05	—	0.26	0.01
	Florida Hard-Rock Phosphate							
932	Dunnellon	35.99	0.01	0.00	None	0.00	0.56	0.55
	Tennessee Brown-Rock Phosphate							
—	Bur. of Standards, standard sample No. 56	31.28	1.24	0.00	Trace	1.19	2.69	0.26
762	Mountpleasant	33.73	0.75	0.00	None	0.00	0.81	0.06
906	Wales	34.39	0.59	0.02	None	0.00	0.71	0.10
908	Mountpleasant	34.44	0.68	0.02	None	0.00	0.76	0.06

Tennessee Blue-Rock Phosphate

No.	Locality							
772	Glover	30.45	4.02	0.06	Trace	2.71	7.10	0.31
930	Gordonsburg	30.97	1.26	0.01	Trace	5.21	6.64	0.16
1049*	Boma	31.22	2.00	—	Trace	2.02	4.07	0.05
448	Glover	32.02	2.43	0.02	Trace	1.37	4.04	0.22
449	Gordonsburg	33.65	1.06	0.00	Trace	3.19	4.37	0.12

Tennessee Phosphatic Limestone

No.	Locality							
916	Mountpleasant	11.22	1.55	—	Trace	0.97	2.57	0.05
917	Gordonsburg	11.68	2.27	—	Trace	2.52	4.84	0.05

Phosphates from Western United States

No.	Locality							
550	Paris, Idaho	32.21	1.43	0.00	0.05	0.00	1.53	0.05
454	Conda, Idaho	32.24	1.66	0.00	0.05	0.00	2.52	0.81
973	Conda, Idaho	32.53	1.69	0.05	0.10	0.00	2.47	0.63
1009	Garrison, Montana	31.39	0.12	0.02	None	0.02	0.33	0.17
1010	Garrison, Montana	37.47	0.26	0.02	None	0.02	0.38	0.08
948	Cokeville, Wyoming	30.19	1.30	0.05	0.09	1.30	3.09	0.35

South Carolina Land-Rock Phosphate

No.	Locality							
1139	Bulow Mines, Johns Island	26.92	1.74	0.00	Trace	0.48	2.51	0.29
1138	Lamb's Mines, Charleston	27.85	1.97	0.00	Trace	0.46	2.65	0.22

North African Phosphate

No.	Locality							
552	Gafsa, Tunis	27.55	2.87	0.07	None	0.00	3.53	0.59
1162	Morocco	35.11	1.40	—	None	0.00	1.46	0.06

* Kidney phosphate.

organic carbon in three samples of Florida hard rock. With the exception of these figures, the available data, so far as the authors are aware, are limited to qualitative results and figures for "organic matter and combined water," as determined from the ignition loss. The phosphates of Idaho, Utah, and Wyoming contain relatively large amounts of organic material, which in some instances is oil-bearing.[1] Nitrogen (N), in amounts ranging from a trace to 0.21 per cent, has been reported in South Carolina phosphates.[2] Data relating to the water content of phosphates seem to be limited to fairly pure specimens of the phosphatic constituent of phosphate rock.

TABLE 7
Acid-insoluble organic sulfur

SAMPLE NUMBER	TYPE OF PHOSPHATE	TOTAL SULFUR MINUS SUM OF FRACTIONAL DETERMINATIONS[a]	ORGANIC SULFUR IN RESIDUE IN-SOLUBLE IN HCl (1+4), AS SO_3[b]	DIFFERENCE
		per cent	*per cent*	*per cent*
617	Florida land pebble	0.12	0.09	−0.03
619	Florida land pebble	0.12	0.10	−0.02
790	Florida land pebble	0.14	0.11	−0.03
627	Florida land pebble	0.21	0.07	−0.14
932	Florida hard rock	0.55	0.45	−0.10
—	Bur. Standards stand. samp. 56	0.26	0.21	−0.05
762	Tennessee brown rock	0.06	0.07	+0.01
906	Tennessee brown rock	0.10	0.04	−0.06
772	Tennessee blue rock	0.31	0.05	−0.26
930	Tennessee blue rock	0.16	0.12	−0.04
448	Tennessee blue rock	0.22	0.13	−0.09
454	Idaho phosphate	0.81	0.79	−0.02
973	Idaho phosphate	0.63	0.72	+0.09
948	Wyoming phosphate	0.35	0.50	+0.15
1009	Montana phosphate	0.17	0.03	−0.14
1139	South Carolina phosphate	0.29	0.18	−0.11
1138	South Carolina phosphate	0.22	0.13	−0.09
552	Tunis phosphate	0.59	0.45	−0.14

[a] Results taken from Table 6.
[b] Eschka sulfur less acid-insoluble sulfates and sulfides.

Organic carbon.—The results for organic carbon (Table 8) show that, of the twenty-nine samples analyzed, those from Conda, Idaho, and Cokeville, Wyoming, are by far the richest in organic matter. Whereas the carbon content of these samples is considerably above 2 per cent, the Tennessee kidney phosphate (No. 1049) is the only other sample which contains more than 1 per cent. The results on the other samples of domestic, island, and Morocco phosphates range from 0.09 to 0.52 per cent.

Nitrogen.—The rock sample was digested according to the official Kjeldahl-Gunning-Arnold method (*Methods of Analysis, A.O.A.C.*, 1930,

[1] U. S. Geol. Survey Professional Paper, 152, 211 (1927).
[2] U. S. Geol. Survey, Mineral Resources for 1882, 504 (1883).

21), and after distillation the ammonia in the distillate was estimated colorimetrically by nesslerization (*ibid.*, 404). The materials richest in carbon (Table 8) contain the larger percentages of nitrogen. Thus, the Idaho and Wyoming phosphates contain approximately 0.1 per cent, and the kidney phosphate 0.25 per cent, in comparison with a range of 0.004–0.051 per cent for all other samples analyzed. As indicated by its light color, phosphate from Paris, Idaho, carries much less organic matter than that from the Conda locality, and it may be noted in this connection that a sample of the Paris phosphate, not included in Table 8, contained only 0.02 per cent of nitrogen.

Total water.—The results for total water in rock phosphates, exclusive of water driven off at 105° C., range from 0.6 per cent in high-grade Montana phosphate to 3.4 per cent in a sample of low-grade Florida hard rock. With the exception of Montana phosphate, Tennessee brown rock contains less water than the other types of phosphate. As would be expected, the Florida soft and waste-pond materials show considerably higher percentages of water than the rock phosphates.

Some idea of the amount of water derived from the organic matter in several types of phosphate may be obtained from the first column of results in Table 1. Inasmuch as the composition of the organic material may be altered considerably as a result of the acid treatment necessary to remove the inorganic constituents, these figures, even where the recovery of carbon is complete, are to be regarded only as approximate values which are most probably low. Considering only the materials on which complete recovery of carbon was obtained by the isolation method, viz., Tennessee brown rock, Tennessee blue rock, and Idaho phosphate, the results are about 0.05, 0.16, and 0.8 per cent water, respectively, in terms of the rock sample. If then, in the case of the Idaho samples, Nos. 454 and 973, the figures in Table 1 can be used to correct the total water for organic hydrogen, the corrected results are 1.11 and 1.13 per cent, respectively, and represent the percentages of water associated with the mineral constituents of these materials. These figures agree quite well with Rogers' result,[1] 1.05 per cent, on the water content of a fairly pure sample of phosphate mineral obtained from phosphate rock from the Crawford Mountains, Utah.

COMPOSITION OF THE ORGANIC MATERIAL OCCURRING IN PHOSPHATE ROCK

The composition of the organic matter separated from air-dried samples of phosphate rock (Table 9) varies within rather wide limits. In particular, the material obtained from Florida hard rock is characterized by relatively low percentages of carbon and nitrogen, and by very high percentages of sulfur and ash. Furthermore, the results on the two samples of

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[1] *Am. J. Sci.*, **3**, 269 (1922).

TABLE 8

Organic carbon, nitrogen, and total water in phosphate rock

SAMPLE NUMBER	SOURCE OF PHOSPHATE	P_2O_5	ORGANIC CARBON	NITROGEN	TOTAL WATER[a]	ORGANIC CARBON, NITROGEN AND WATER	
						BY ANALYSIS	IGNITION LOSS MINUS CO_2
		per cent	per cent	per cent	per cent	per cent	per cent
	Florida Land-Pebble Phosphate						
619	Nichols	30.98	0.33	0.018	2.27	2.62	2.00
910[b]	Mulberry	31.09	0.33	0.006	2.57	2.91	2.84
947	Brewster	31.28	0.29	0.008	2.49	2.79	2.29
439	Mulberry	33.22	0.25	—	1.88	2.13	1.89
912[b]	Mulberry	35.37	0.38	0.013	1.35	1.74	1.62
	Florida Hard-Rock Phosphate						
771	Unknown	31.25	0.52	0.018	3.40	3.94	2.96
932[b]	Dunnellon	35.99	0.18	0.005	1.48	1.67	1.00
	Florida Soft Phosphate						
728[b]	Juliette	31.80	0.16	0.018	3.85	4.03	3.88
	Florida Waste-Pond Phosphate						
726[b]	Felicia	23.48	0.22	0.024	5.81	6.05	5.64
915	Dunnellon	23.63	0.47	0.044	8.18	8.69	9.34
	Tennessee Brown-Rock Phosphate						
	Bureau of Standards standard sample						
—	No. 56	31.28	0.25*	0.023	1.66*	1.93	1.86
762	Mountpleasant	33.73	0.29	0.015	1.39	1.70	1.32
906[b]	Wales	34.39	0.09	0.006	1.29	1.39	2.03
908[b]	Mountpleasant	34.44	0.11	0.004	1.31	1.42	1.26

Tennessee Blue-Rock Phosphate								
772	Glover	30.45	0.36*	0.034	1.84*		2.23	2.87
930b	Gordonsburg	30.97	0.20*	0.021	1.06*		1.28	1.19
1049b,d	Boma	31.22	1.46*	0.26	2.42*		4.14	4.11
Tennessee White-Rock Phosphate								
1048b	Tomscreek	30.20	0.09	0.016	1.55		1.66	0.80
1031b	Godwin	35.80	0.26	0.033	2.53		2.82	2.43
Phosphates from Western United States								
454	Conda, Idaho	32.24	2.29	0.091	1.96		4.34	4.55
973c	Conda, Idaho	32.53	2.35	0.10	1.90		4.35	4.17
1009b	Garrison, Montana	31.39	0.14	0.008	1.10		1.25	1.07
1010b	Garrison, Montana	37.47	0.09	0.008	0.60		0.70	0.37
948b	Cokeville, Wyoming	30.19	2.69*	0.11	1.50*		4.30	3.39
South Carolina Land-Rock Phosphate								
1138	Lamb's Mines, Charleston	27.85	0.51*	0.051	3.31*		3.87	3.84
North African Phosphates								
552	Gafsa, Tunis	27.55	0.86	0.047	3.05		3.96	3.91
1162	Morocco	35.11	0.17	0.018	1.44		1.63	1.77
Island Phosphates								
985	Curaçao Island	38.59	0.17	0.013	2.29		2.47	1.02
904b,f	Connetable Islands	54.51	0.28	0.049	1.68		2.01	1.97

a Results do not include moisture given off at 105° C.
b Air-dried material.
c Crushed rock was dried in rotary kiln with coal fire.
d Kidney phosphate.
e Determination was made at 600° C.
f Hydrated aluminum phosphate.

Florida land pebble indicate that the composition, especially the carbon content, of the organic material from different samples of the same type of phosphate may also be subject to considerable variation. However, if the sample of Florida hard rock be excluded on account of its extremely high ash, the carbon results on the other materials may be combined in a simple average. Thus, considering the results (not ash-free) on the organic material from two samples of Florida land pebble and from one each of Tennessee brown rock and phosphate from Conda, Idaho, the average percentage of carbon is 59.6. In view of the paucity of results further discussion of the composition is not justifiable.

TABLE 9

Composition of organic material isolated from phosphate rock[a]

SAMPLE NUMBER	TYPE OR SOURCE OF PHOSPHATE	WT. OF ROCK USED IN SEPARATION	COLOR OF ORGANIC MATERIAL	ELEMENTARY ANALYSIS				
				ASH[b]	C	H	N	S
		grams		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
910	Florida land pebble	200	dark brown	2.50	63.3	5.26	3.41	7.32
912	Florida land pebble	200	dark brown	12.6	53.1	4.39	1.88	10.2
932	Florida hard rock	400	brownish black	50.2	22.6	2.08	0.16	39.1
908	Tennessee brown rock	400	dark brown	9.53	60.8	3.41	3.15	8.11
973[c]	Idaho	30	black	2.32	61.3	2.62	2.07	7.90

[a] The procedure of Fieldner, Selvig and Taylor (*Loc. cit.*) was used in making these large-scale separations, most of the quarts being removed by decantation. The isolated organic material was dried at 105° C.
[b] Principally iron oxides.
[c] Crushed rock was dried in a rotary kiln with coal fire; other samples were air-dried.

SUMMARY

Results are presented for total sulfur, acid-soluble and acid-insoluble sulfate, and acid-soluble and acid-insoluble sulfide in twenty-six samples of domestic rock phosphate of commercial grade from various localities, two samples of phosphatic limestone, and two samples of North African phosphate. Sulfur was found in all samples analyzed.

The organic carbon, as determined by dry combustion of the rock, was used as a standard measure of the amount of organic matter present in the phosphates. In addition, comparative data were obtained by several other methods, viz. (1) organic carbon by dry combustion of the organic material actually separated from the rock, (2) organic matter by a colorimetric control method, (3) organic matter and water by calculation from the ignition loss. Quantitative data on the several factors which affect the ignition loss are also given.

Results are given for organic carbon, nitrogen, and total water in twenty-nine samples of natural phosphates from various deposits in the United States and elsewhere. Large-scale separations of the organic material were made on several types of phosphate rock, and the isolated materials were analyzed for carbon, hydrogen, nitrogen, and sulfur.

DETERMINATION OF INACTIVE MALIC ACID IN FRUITS AND FRUIT PRODUCTS

By B. G. Hartmann and F. Hillig (Food Control,[1] Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

The literature does not record the occurrence of inactive malic acid in fruits, and the results obtained in this investigation justify the conclusion that this acid is not a normal constituent of fruits.

As inactive malic acid is now quite generally employed in the preparation of fruit products, a method for its determination was considered necessary. Therefore, the investigation described was undertaken.

Inactive malic acid is now available in the market in practically pure condition and at low cost. It is prepared synthetically by the catalytic oxidation of benzene to maleic acid and conversion of the latter into malic acid by heating with water under pressure. The commercial article, commonly referred to as synthetic malic acid, is a white crystalline compound having an empyreumatic odor. Chemically it is the racemic form of malic acid, composed of equal parts of *l* and *d* malic acid. It forms insoluble lead compounds and in general behaves like laevo malic acid toward solvents. As the name implies, the acid does not turn the plane of polarized light, a characteristic which definitely distinguishes it from fruit malic acid, which turns the light to the left.

The method for the determination of inactive malic acid proposed involves (1) the isolation of total malic acid (laevo and inactive modifications), (2) the determination of the total malic acid by oxidation with potassium permanganate in alkaline solution, and (3) the determination of the inactive malic acid by subtracting the laevo acid obtained polarimetrically from the total malic acid.

The writers have published[2] a procedure for the isolation and determination of laevo acid in fruits and fruit products, in which it is not necessary to carry the purification of the isolated material beyond the removal of interfering optically active substances. Because, as stated before, the procedure for *inactive* malic acid involves the determination of total malic acid by an oxidative reaction, it is necessary not only to remove the interfering optically active substances, but to extend the purification to the exclusion of all oxidizable substances not malic acid.

For the isolation of total malic acid the general procedure described for laevo malic acid was followed, but in order to reduce the interfering substances to a minimum an additional step, precipitation with lead ace-

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tate just prior to the removal of cream of tartar, was introduced. Although this adjustment does not entirely eliminate interfering substances, it was found that by means of it the total malic acid isolated from fruits was made practically uniform with regard to included non-malic impurities. These consist of small quantities of citric, tartaric, and succinic acids and tannins, and with the exception of succinic acid all are oxidizable with permanganate in alkaline solution.

In the experimental work done in developing the method presented the commercial inactive malic acid without purification was used because the problem was to devise a procedure for determining *commercial* inactive malic acid rather than the chemical entity.

EXPERIMENTAL

In Table 1 are given the data for determining the non-malic impurities remaining in the isolated aliquot. The aliquots "A" and "B" were isolated from nine common fruit products by the procedure described. "A" represents milligrams of total oxidizable material as malic (cc. $KMnO_4 \times 5$), and "B" represents laevo malic acid ($V° \times 10.2$). Column "C" records the milligrams of oxidizable non-malic impurities present in the aliquot.

TABLE 1

Determination of non-malic impurities in the isolated aliquot

MATERIAL	A	B	C
	TOTAL OXIDIZABLE MATERIAL AS MALIC	LAEVO MALIC ACID (POLARI-METRICALLY)	NON-MALIC IMPURITIES A−B
	mg.	*mg.*	
Commercial grape juice	16.3	12.2	4.1
Strawberry jam	11.5	5.1	6.4
Apple jam	23.8	18.4	5.4
Blackberry jam	8.0	2.0	6.0
Peach jam	10.1	6.1	4.0
Orange juice	7.0	2.0	5.0
Red raspberry jam	6.3	1.0	5.3
Damson plum jam	55.3	51.0	4.3
Prune jam (Oregon, Italian style)	45.0	40.8	4.2
		Average	5.0

The data in Column "C" show that under the conditions maintained in the isolation procedure approximately 5 mg. of impurities passes into solution. The figures for the individual determinations vary slightly, but when the wide differences in the acid constituents of the nine fruit products represented are considered, they are entirely satisfactory.

Table 2 presents the data for deriving the factor 10.2 for converting degrees Ventzke to milligrams of laevo malic acid. The data were obtained on aqueous solutions of laevo malic acid. The solutions were treated with uranium acetate under the conditions described in the proposed procedure. It was found that the presence of inactive malic acid and the small quantities of non-malic acid (citric, tartaric and succinic acids) remaining in the isolated aliquot do not affect the polarimetric measurements.

TABLE 2

Determination of the factor 10.2

LAEVO MALIC ACID PRESENT	POLARIMETRIC READING	LAEVO MALIC ACID
mg.	°*V.*	°*V.*
19.8	1.95	10.15
39.6	3.90	10.15
59.0	5.80	10.17
78.9	7.70	10.25
98.6	9.65	10.22
	Average	10.2

The milligrams of corrected inactive malic acid (i.A.) in the aliquot are obtained by the following formula:

i.A. = T − 5 − L, in which

T = mg. of total oxidizable material as malic,

5 = mg. of non-malic material as malic,

L = mg. of laevo malic acid.

Table 3 presents experimental data for deducing the factor for reverting "i.A." back to the milligrams of inactive malic acid contained in the portion taken for analysis. For obtaining this factor the fruit products listed in Table 1 were used. After the addition of inactive malic acid two portions of the material were subjected to the isolation procedure. In one of the aliquots the laevo malic acid was determined, and in the other the total oxidizable material was obtained. The quantity of added inactive acid in the portion taken for analysis divided by the corrected inactive malic acid (i.A.) in the aliquot represents the reversion factor.

Notwithstanding the wide variation in the acid constituents of the fruits used, the factor is remarkably uniform, ranging from 3.85 to 4.12 with an average of 4.00. This factor, as well as all the other factors described, is empirical and is applicable only under strict observance of all the conditions laid down in the isolation procedure. The substitution of volumetric flasks of different capacity than those indicated is not permissible.

It will be noted that the conditions in the isolation procedure for laevo malic acid described previously[1] differ from those prescribed for isolating

TABLE 3

Factor for converting inactive malic acid in the aliquot to original

| MATERIAL | L-MALIC ACID | | TOTAL OXIDIZABLE MATERIAL AS MALIC IN ALIQUOT | LAEVO MALIC ACID IN ALIQUOT | OXIDIZABLE MATERIAL NOT MALIC IN ALIQUOT[*] | REVERSION FACTOR $\frac{A}{B}$ |
	A IN ORIGINAL PORTION TAKEN FOR ANALYSIS	B IN ALIQUOT				
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	
Grape juice	98.5	25.6	34.8	5.1	4.1	3.85
Strawberry jam	98.5	24.1	32.5	2.0	6.4	4.09
Apple jam	97.8	24.2	52.0	22.4	5.4	4.04
Blackberry jam	98.5	23.9	33.0	3.1	6.0	4.12
Peach jam	96.5	23.8	36.0	8.2	4.0	4.05
Orange juice	97.8	25.0	31.0	1.0	5.0	3.91
Red raspberry jam	97.8	24.2	30.5	1.0	5.3	4.04
Damson plum jam	97.8	24.2	52.0	23.5	4.3	4.04
Prune jam (Oregon, Italian style)	99.5	25.4	50.0	20.4	4.2	3.92

Average 4.0

[*] Taken from Table 1.

TABLE 4

Effect of precipitated lead salts on recovery of l-malic acid

SOLUTIONS CONTAINED				LAEVO MALIC ACID	
CITRIC ACID	TARTARIC ACID	INACTIVE MALIC ACID	LAEVO ACID	FOUND IN ALIQUOT	RECOVERY
mg.	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*
100	0	25	24.8	5.1	20.6
25	75	—	49.8	11.2	22.5
50	25	—	77.1	19.4	25.2
25	50	50	24.8	6.1	24.6
50	0	—	99.8	27.5	27.6
25	25	25	49.9	14.3	28.7
25	0	25	77.1	25.5	33.1
0	25	—	123.3	39.8	32.3
0	0	100	49.9	17.3	34.7
0	0	50	99.8	34.1	34.2
0	0	25	123.3	43.9	35.6

the total malic acid in the proposed method. Citric acid and tartaric acid are added for the purpose of adjusting the isolated aliquot to a uniform content of oxidizable non-malic material. It is obvious that the correction factor for non-malic material is not applicable to fruit products that are

[1] *This Journal,* 15, 647 (1932).

free of these acids and it is necessary, therefore, to provide for their presence by a deliberate addition. It was found that the introduction of the extra step in the malic acid procedure causes an occlusion of laevo malic acid, and that the occlusion is directly proportional to the quantity of precipitated lead salts, so that the recoveries of malic acid are not constant. This condition is shown in the last column of Table 4.

The data in Table 4 were obtained by subjecting aqueous solutions containing known quantities of laevo malic acid in admixture with other acids to the isolation procedure of the proposed method.

The recoveries of malic acid vary so widely that it is evident that the isolation procedure proposed is not suitable for obtaining laevo malic acid.

The following procedure for the determination of inactive malic acid (commercial product) in fruits and fruit products resulted from this study.

METHOD

Inactive malic acid in fruits and fruit products

The inactive malic acid is represented by the difference between the total malic acid and the laevo malic acid, and therefore it is necessary to run the isolation procedure in duplicate.

PREPARATION OF SAMPLE

Do not allow the acidity (as malic acid) of the portion taken for analysis to exceed 150 mg., and in no case have the *solids* content materially exceed 20 grams (200 cc. of the sample solution of a jam or jelly).

Determine the acidity of the portion taken in terms of normal acid and designate as "A." Adjust to a volume of about 35 cc., either by evaporation or by the addition of water. Pour the contents of the beaker into a 250 cc. volumetric flask and rinse the beaker with 10 cc. of warm water and finally with 95 per cent alcohol; cool, make to mark with 95 per cent alcohol, shake, and filter through an S & S No. 588 folded filter paper, 18½ cm. During filtration cover the funnel with a watch-glass to retard evaporation of alcohol. Transfer 225 cc. of the clear filtrate to a 16-ounce centrifuge bottle (narrow mouth tincture).

In case the product contains esters (indicated by the presence of alcohol) saponify the esters by adjusting the volume to about 35 cc., add "A" plus 3 cc. of normal *potassium* hydroxide, heat to about 60° C., and allow to stand overnight. Add a quantity of normal sulfuric acid equivalent to "A" plus 3. Pour the mixture into a 250 cc. volumetric flask, rinse the beaker with 10 cc. of warm water and finally with 95 per cent alcohol, cool, and make to mark with the alcohol. Filter, and transfer 225 cc. of the clear filtrate to a 16-ounce centrifuge bottle.

REAGENTS

Lead acetate solution.—Dissolve 40 grams of lead acetate, $Pb(CH_3COO)_2 \cdot 3 H_2O$, in water, add 0.5 cc. of glacial acetic acid, and make to 100 cc.

Standard tribasic lead acetate solution.—Prepare the solution from the tribasic lead acetate described below. To 5 grams of the salt in a 500 cc. Erlenmeyer flask, add 200 cc. of distilled water and shake vigorously. Neutralize 3 cc. of normal sulfuric acid, diluted with 200 cc. of water, with the lead solution, using methyl red as

indicator. Note the volume of the lead solution required. In the determination use 2 cc. in excess of this quantity. The solution should be freshly prepared.

Tribasic lead acetate.—Dissolve 82 grams of lead acetate $Pb(CH_3COO)_2 \cdot 3 H_2O$ in 170 cc. of distilled water. Prepare 100 cc. of dilute ammonium hydroxide containing 5.8 grams of ammonia (NH_3) as determined by titration (methyl red). Heat the solutions to 60° C., mix thoroughly, and allow to stand overnight. Shake vigorously to break up the precipitate, and filter on a Büchner funnel; wash once with water and suck dry, then twice with 95 per cent alcohol, and finally with ether. Allow to dry in air.

Potassium permanganate solution.—Dissolve 14.5214 grams of the purest potassium permanganate in distilled water and dilute to 1 liter. Standardize the solution as follows: Pipet 50 cc. of the oxalic acid solution into a 600 cc. beaker and add 70 cc. of distilled water and 10 cc. of sulfuric acid (1+1). Heat to 80° C., immediately run in the permanganate solution until a faint pink color is produced, again heat to 80°C., and finish the titration. Fifty cc. of the permanganate solution should equal 50 cc. of the oxalic acid solution. One cc. of the permanganate solution is equivalent to 5 mg. of malic acid (laevo or inactive).

Oxalic acid solution.—Dissolve 28.7556 grams of the purest oxalic acid in distilled water and dilute to 1 liter.

Sodium hydroxide solution.—Dissolve about 60 grams of sodium hydroxide in distilled water and dilute to 200 cc.

DETERMINATION

To the material in the centrifuge bottle, add about 25 mg. of citric acid and a quantity of the lead acetate solution equal to "A" (or, in the case saponification was necessary, "A" plus 3) and shake vigorously for 2 minutes. Wash the sides of the bottle with a small quantity of 95 per cent alcohol and centrifugalize at about 900 r.p.m. for 15 minutes. Carefully decant the supernatant liquid and test it with a small quantity of the lead acetate solution; if a precipitate is formed, return to the centrifuge bottle, add more lead acetate, shake, and again centrifugalize. If the sediment lifts repeat the centrifugalizing, increasing the speed and time. Allow the precipitate to drain thoroughly by inverting the bottle for several minutes. Add 250 cc. of 80 per cent alcohol and shake vigorously; again centrifugalize, decant, and drain. Add about 150 cc. of water to the lead salts, shake vigorously, and pass in a rapid stream of hydrogen sulfide to saturation. Stopper the bottle and shake for about 1 minute. Transfer the mixture to a 250 cc. volumetric flask with water, make to mark, shake, and filter. Pipet 225 cc. of the filtrate into a 600 cc. beaker and evaporate to about 100 cc. to expel hydrogen sulfide. Transfer to a 250 cc. volumetric flask with water. (The volume in the flask should be about 200 cc.) Add 5 cc. of a 10 per cent acetic acid solution and the same quantity of lead acetate solution previously used. Shake vigorously, make to mark with water, and filter. Pass a rapid stream of hydrogen sulfide into the *clear* filtrate to saturation, stopper the flask, shake vigorously, and filter. Transfer 225 cc. of the filtrate to a 600 cc. beaker, add about 75 mg. of tartaric acid, and evaporate on a gauze to about 50 cc. Cool, neutralize with normal *potassium* hydroxide (phenolphthalein) and add 5 drops in excess. Add 2 cc. of glacial acetic acid and transfer the mixture to a 250 cc. volumetric flask with 95 per cent alcohol. Make to mark with the alcohol, shake, and pour into a 500 cc. Erlenmeyer flask. Add a small handful of glass beads and cool to 15° C. Stopper the flask, shake vigorously for 10 minutes, and place in the refrigerator for one-half hour. Again shake for 10 minutes and filter through a folded filter paper. Adjust the clear filtrate to 20° C. and transfer 225 cc. to a 16-ounce centrifuge bottle. Add lead acetate solution equal to "A" ("A" plus 3, in the case of saponification), shake vigorously for about 2 minutes, centrifugalize,

decant, and drain. Add 250 cc. of 80 per cent alcohol, shake, centrifugalize, decant, and drain. Transfer the lead salts to a 500 cc. Erlenmeyer flask with about 175 cc. of water. Add 3 cc. of normal sulfuric acid and heat to boiling; add 1 cc. of acetic acid solution (5 cc. of glacial acetic acid made to 100 cc.) and the quantity of the standard tribasic lead acetate solution previously determined under "Reagents." Boil the mixture for 5 minutes, cool to room temperature, transfer to a 250 cc. volumetric flask with water, make to mark, shake, and pour into a 500 cc. Erlenmeyer flask. Add a small handful of glass beads, cool to 15° C., shake vigorously for 5 minutes, and place in the refrigerator for one-half hour. Again shake for 5 minutes and filter through a folded filter paper. Decompose the *clear* filtrates with hydrogen sulfide, shake vigorously, and filter. Use one of the two portions for polarization and the other for oxidation.

For the polarization evaporate 225 of the clear filtrate over a gauze to about 10 cc., neutralize with normal potassium hydroxide (phenolphthalein), make slightly acid with dilute acetic acid, and evaporate to about 5 cc. Transfer to a 27.5 cc. "Giles" flask with water, make to mark, shake, and pour into a small Erlenmeyer flask. Add a small handful of glass beads and 4 grams of uranium acetate, shake vigorously for 10 minutes, and filter. (The uranium-malic complex is sensitive to light, therefore while shaking wrap the flask in a towel and protect from light as much as possible while filtering and polarizing.) Polarize in a 200 mm. tube at 20° C., using white light. After the tubes have been filled, release the tension on the glass disks by slightly loosening the caps, and allow them to remain at 20° C. for at least one-half hour before making the readings. The Ventzke reading multiplied by the factor 10.2 equals the mg. of l-malic acid contained in the aliquot.

For the oxidation evaporate 225 cc. of the remaining portion to about 10 cc. to expel alcohol, make to about 120 cc. with distilled water, and add 10 cc. of the sodium hydroxide solution and 25 cc. of the potassium permanganate solution. Heat to about 75° C. and place in a boiling water bath for one-half hour. Add 25 cc. of the oxalic acid solution and 10 cc. of sulfuric acid (1+1), stirring vigorously. Adjust the temperature to 80° C. and titrate to faint pink color with the permanganate solution. Again heat to 80° C. and finish the titration. The quantity of permanganate used multiplied by 5 equals the total oxidizable material (as malic acid) present in the aliquot.

The inactive malic acid (mg.) in the portion taken for analysis is obtained by the following formula: $X = 4(T - 5 - L)$, in which

4 = factor for reverting the inactive malic acid in the aliquot back to the inactive malic acid in the portion taken for analysis,

T = total oxidizable material (mg.) as malic in the aliquot.

5 = quantity of non-malic material (mg.) as malic in the aliquot; and

L = laevo malic acid (mg.) in the aliquot.

The results obtained by the method are too high by about 0.01 gram per 100 grams material, so that a correction for that quantity of inactive acid must be made.

Note.—The method is empirical, therefore all the directions must be rigidly followed, particularly with respect to dilutions. The substitution of volumetric flasks of a different capacity than that indicated is not permissible.

The proposed method was applied to a number of fruit products. The results are given in Table 5.

The quantity of inactive malic acid added to the sample solution of the fruit is indicated in the second column. The portion taken for analysis then had an acidity of approximately 150 mg. as malic. In some cases the

added inactive acid far exceeded the quantity used in practice. The data on the straight fruits (no acid added) show a quantity of determined inactive acid ranging from −0.02 to +0.02 gram per 100 grams of the fruit

TABLE 5

Added inactive malic acid in fruits and fruit products

MATERIAL	QUANTITY TAKEN FOR ANALYSIS	INACTIVE MALIC ACID		ERROR	IN ALIQUOT	
		ADDED	DETERMINED		CORRECTED TOTAL MALIC ACID	LAEVO MALIC ACID
	grams	*mg.*	*per cent*	*per cent*	*gram per 100 grams*	*mg.*	*mg.*
Commercial grape juice	21.4	—	—	−0.02	−0.02	11.3	12.2
Commercial grape juice	16.1	24.6	0.15	0.12	−0.03	12.0	7.1
Commercial grape juice	5.4	98.5	1.82	1.83	+0.01	29.8	5.1
Strawberry jam	30.0	—	—	+0.02	+0.02	6.5	5.1
Strawberry jam	7.5	98.5	1.31	1.30	+0.01	27.5	3.1
Apple jam	30.0	—	—	+0.01	+0.01	18.8	18.4
Apple jam	30.0	23.4	0.08	0.08	0.00	23.3	17.3
Apple jam	30.0	97.8	0.33	0.33	0.00	47.0	22.4
Blackberry jam	30.0	—	—	+0.01	+0.01	3.0	2.0
Blackberry jam	30.0	24.6	0.08	0.07	−0.01	8.0	3.1
Blackberry jam	15.0	98.5	0.66	0.66	0.00	28.0	3.1
Peach jam	30.0	—	—	−0.02	−0.02	4.8	6.1
Peach jam	30.0	24.1	0.08	0.07	−0.01	13.3	8.2
Peach jam	22.5	96.5	0.43	0.41	−0.02	31.0	8.2
Orange juice	10.0	—	—	0	0.00	2.0	2.0
Orange juice	9.0	24.5	0.27	0.24	−0.03	6.5	1.0
Orange juice	7.5	48.9	0.65	0.61	−0.04	12.5	1.0
Orange juice	5.0	73.4	1.47	1.38	−0.09	18.3	1.0
Red raspberry jam	30.0	—	—	0	0.00	1.3	1.0
Red raspberry jam	22.5	48.9	0.22	0.21	−0.01	12.8	1.0
Red raspberry jam	15.0	97.8	0.65	0.65	0.00	25.5	1.0
Damson plum jam	16.5	—	—	−0.02	−0.02	50.3	51.0
Damson plum jam	11.3	48.9	0.43	0.38	−0.05	48.5	37.7
Damson plum jam	6.0	97.8	1.63	1.57	−0.06	47.0	23.5
Prune jam (Oregon Italian style)	11.3	—	—	+0.01	+0.01	40.0	40.3
Prune jam (Oregon Italian style)	9.0	49.7	0.55	0.56	+0.01	47.0	35.7
Prune jam (Oregon Italian style)	4.5	99.5	2.21	2.19	−0.02	45.0	20.4
Commercial raspberry sirup	15.0	—	—	0.84		33.5	2.0
Commercial cherry sirup	15.0	—	—	0.81		38.5	8.2

product. Of the 18 fruit samples containing added inactive malic acid, four show no error, eleven show a minus error, and three show an error of +0.01 per cent. Based upon these determinations, in the interpretation

of the results obtained by the method, a correction of minus 0.01 gram per 100 grams material should be made.

SUMMARY

A procedure is presented for the determination of inactive malic acid in fruits and fruit products. It involves (1) isolation of total malic acid (laevo and inactive modifications), (2) determination of the total malic acid by oxidation with potassium permanganate in alkaline solution, and (3) determination of the inactive malic acid by subtracting the laevo acid obtained polarimetrically from the total malic acid. The procedure is easy to follow, but it is time-consuming. In the interpretation of the results obtained by the method it is necessary to make a correction of 0.01 per cent.

DETERMINATION OF SMALL QUANTITIES
OF BENZYL ALCOHOL

By Joseph Callaway, Jr., and Solomon Reznek (U. S. Food and Drug Administration, New York, N. Y.)

Benzyl alcohol has a definite, if limited, use as a drug. It and the esters occur in some of the essential oils, and some of its esters, notably benzyl benzoate and, lately, benzyl fumarate and benzyl succinate are regularly, if not extensively used as pharmaceuticals. The identification and quantitative determination of benzyl esters depend upon the determination of the benzyl radical. Finding no available method in the literature, the authors developed the quantitative procedures described. The investigation was confined largely to the determination of benzyl alcohol in water solution and in dilute (physiological) salt solution, since benzyl alcohol is usually dispensed in such solutions.

All the benzyl alcohol used was obtained by redistilling the commercially pure product and separating that portion distilling between 205.8 and 206.3°C. at atmospheric pressure. It had a specific gravity at 25/25° C. of 1.0488, and the refractive index at 24° C. was 1.5385.

Studies were made to determine the solubility of benzyl alcohol in water, saturated salt solution, ethyl alcohol, ether, petroleum ether, and other organic solvents. It was found to be miscible with all the organic solvents tried except petroleum ether, in which it is only slightly soluble. The solubility of benzyl alcohol in water has been reported by different investigators as being about 4 parts of benzyl alcohol to 100 parts of water. It was found that a solution of this concentration can be effected with difficulty, that such a solution is cloudy, and that saturation with salt reduces the solubility materially.

The immersion refractometer readings and specific gravity of solutions of varying strengths of benzyl alcohol in water were determined (Table 1).

The cloudiness of the solution made it impracticable to obtain accurate readings on solutions containing more than 3 grams of benzyl alcohol per 100 cc.

Plotting the refractometer reading against the amount of benzyl alcohol present gives a straight line. Therefore, the amount of benzyl alcohol in a water solution can be calculated from the immersion refractometer reading by the following simple formula calculated from the data in the table:

Grams of benzyl alcohol/100 cc. of solution = $(r - 14.40) \times 0.193$, where "r" is the refractometer reading of 20.0° C. and 14.40 is the reading for distilled water at 20.0° C.

The specific gravities of aqueous solutions of benzyl alcohol are too close to unity and vary too slightly to allow accurate computation of the quantity of benzyl alcohol from the specific gravity. However, a specific

TABLE 1
*Immersion refractometer and specific gravity readings of aqueous solutions
of benzyl alcohol*

GRAMS/100 CC. OF BENZYL ALCOHOL	SPECIFIC GRAVITY 20°/20°	SCALE READING AT 20°C.
0.00	1.0000	14.40
0.01	1.0000	14.45
0.05	1.0000	14.65
0.10	1.0001	14.90
0.25	1.0002	15.70
0.40	1.0003	16.45
0.50	1.0004	16.90
0.80	1.0006	18.60
1.00	1.0006	19.55
1.50	1.0010	22.15
2.00	1.0014	24.8
2.51	1.0016	27.3
3.00	1.0021	29.9

gravity determination in conjunction with a refractometer reading is of value to show the presence or absence of substances other than benzyl alcohol. If other substances are not present, the proportion of benzyl alcohol may be calculated from the refractometer reading.

Water solutions of benzyl alcohol of varying concentrations were examined as follows: 100 cc. portions were diluted to 110 cc. and distilled and the first 100 cc. of distillate was collected. Immersion refractometer readings showed recoveries of from 91.4 to 98.4. By adding an additional 50 cc. of water to the residual portion and collecting an additional 50 cc. of distillate, the total recovery was raised to an average of 99.0. By again adding 50 cc. of water and collecting 50 cc. of distillate, practically complete recovery was possible.

TABLE 2

Results of varying concentration of permanganate, time and temperature of oxidation, and the quantity of benzyl alcohol

CONCENTRATION OF PERMANGANATE	TIME OF OXIDATION	TEMPERATURE OF OXIDATION	BENZOL ALCOHOL TAKEN	BENZOL ALCOHOL FOUND	YIELD
per cent	*hours*		*gram*	*gram*	*per cent*
0.5	2	Room	0.0994	0.0945	95.1
0.5	2	Room	0.0994	0.0945	95.1
1.0	2	Room	0.0994	0.0964	97.0
1.0	2	Room	0.0994	0.0931	93.7
3.0	2	Room	0.0994	0.0926	93.2
3.0	2	Room	0.0994	0.0930	93.6
5.0	2	Room	0.0994	0.0944	95.0
5.0	2	Room	0.0994	0.0936	94.2
1.0	½	Room	0.0888	0.0822	92.6
1.0	½	Room	0.0888	0.0825	92.9
1.0	1	Room	0.1083	0.1030	95.1
1.0	1	Room	0.1218	0.1163	95.5
1.0	2	Room	0.0888	0.0826	93.0
1.0	2	Room	0.0888	0.0818	92.1
1.0	24	Room	0.0888	0.0842	94.8
1.0	24	Room	0.0888	0.0834	93.9
1.0	½	Steam bath	0.1152	0.1077	93.5
1.0	½	90°C.	0.1234	0.1165	94.4
1.0	1	90°C.	0.0888	0.0830	93.5
1.0	1	90°C.	0.0888	0.0813	91.6
1.0	2	90°C.	0.0888	0.0769	86.6
1.0	2	90°C.	0.0888	0.0787	88.6
1.0	1	Room	0.0053	0.0058	109.4
1.0	1	Room	0.0053	0.0058	109.4
1.0	1	Room	0.0106	0.0104	98.1
1.0	1	Room	0.0106	0.0094	88.7
1.0	1	Room	0.0502	0.0469	93.4
1.0	1	Room	0.0502	0.0452	90.0
1.0	1	Room	0.2074	0.1943	93.7
1.0	1	Room	0.2088	0.1964	94.1
Blanks			—	0.0005	
				0.0005	1 drop 0.1 N
				0.0005	alkali

Portions of 100 cc. solutions of benzyl alcohol in water containing 1.0–1.5–2.0 grams per 100 cc. were steam-distilled by the official method for anthranilic esters.[1] In order to obtain a complete recovery of benzyl alcohol it was necessary to collect from 300 to 400 cc. of distillate, the larger quantity being necessary for the higher concentrations. Steam-distillation

[1] *Methods of Analysis, A.O.A.C.*, 1930, 132.

requires about the same time as the triple distillation method described. However, the triple distillation method is considered preferable as it is desirable to restrict the volume of the distillate.

The method given below is based on the characteristic reaction of benzyl alcohol, whereby it is oxidized to benzoic acid.

DETERMINATION OF BENZYL ALCOHOL

(Applicable to aqueous solutions containing no volatile substances
other than the benzyl alcohol)

Place a quantity of sample corresponding to not more than 3 grams or not less than 0.5 gram of benzyl alcohol into a 500 cc. round-bottomed flask. Add water to make up to 110 cc., attach a Reichert-Meissl stillhead and distil down to about 20 cc., collecting the distillate in a 200 cc. volumetric flask. Cool the distillation flask, add 50 cc. of water to the residue, and distil an additional 50 cc. into the volumetric flask. Again cool, add 50 cc. of water, and collect an additional 50 cc. of distillate. If the benzyl alcohol separates into droplets, which do not go into solution or a uniform suspension on vigorous shaking, repeat the procedure, using a smaller quantity of sample. Make up the distillate to 200 cc. with water.

Determine the specific gravity of the distillate at 20°/20° C. and the immersion refractometer reading at 20.0° C. If concordant results for benzyl alcohol are obtained, showing the absence of other volatile substances, the quantity calculated from the refractometer reading may be considered accurate. (If desired, the result may be verified by oxidizing a portion of the distillate and determining benzoic acid as described below.)

Place an aliquot containing from 0.050 to 0.175 gram of benzyl alcohol in a 500 cc. Erlenmeyer flask, and add 10 cc. of saturated potassium permanganate solution and sufficient water to make the final volume about 60 cc. Securely stopper the flask with a rubber stopper and allow it to stand 1 hour at room temperature, shaking occasionally. After completion of the oxidation remove the stopper and wash down stopper and neck of flask with water. Decolorize with 5 cc. of saturated sodium sulfite solution and 1 cc. of concentrated sulfuric acid. Transfer to a separatory funnel, rinsing the flask with a little water, and add sufficient salt to saturate. Avoid an excess. If any salt remains undissolved, add water in small portions until dissolved by vigorous shaking. Shake out successively with one 50 cc. and three 25 cc. portions of chloroform. Allow to stand until the chloroform layer is quite clear and then filter through a loose plug of cotton into a 250 cc. fat flask. Distil the chloroform over a water bath at 75° C. until approximately 30 cc. remains. Disconnect from the condenser and insert a two-holed stopper containing glass tubes bent at right angles and extending a short distance below the stopper. Support the flask by a clamp to a suitable stand and immerse it in water at a temperature not exceeding 40° C., to prevent condensation and to hasten evaporation. Draw air through one tube, lightly plugging the other with cotton to exclude dust, at such a rate as not to splash the chloroform. Evaporate to dryness and place in a desiccator overnight. Dissolve in 25 cc. of alcohol neutralized to phenolphthalein and titrate with 0.10 *N* alkali. One cc. of 0.10 *N* alkali = 0.0108 gram of benzyl alcohol.

The results obtained show that benzyl alcohol in water or dilute salt solution can be determined quantitatively with a fair degree of accuracy by oxidation to benzoic acid. In the absence of all volatile substances except water, a more accurate determination may be made from the immer-

sion refractometer reading of the combined distillates as described heretofore.

SUMMARY

A study was made of some of the physical properties of benzyl alcohol and of conditions affecting the yield of benzoic acid obtained by its oxidation. A method is proposed for determining small quantities of benzyl alcohol in aqueous solutions.

DETERMINATION OF URONIC ACIDS AND METHOXYL IN CERTAIN PLANTS AND PLANT MATERIALS[1]

By Max Phillips, M. J. Goss, and C. A. Browne (Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.)

The uronic acids are polyhydroxy aldehydic. These acids, together with the polymerized anhydrides or polyuronides, are of almost universal occurrence in the plant world. They serve as structural components of the cell wall and play an important role in phyto-physiological processes.

In the conventional methods for the analysis of plants and materials of vegetable origin, no provision is made for the determination of the percentage of uronic acids. This is especially surprising, in view of the fact that there are fairly accurate methods for the determination of uronic acids.

The work described in this paper, which is of a preliminary nature, was undertaken for the purpose of determining the percentage of uronic acids in certain food plants. Besides throwing additional light on the composition of these plants and plant materials these data may be helpful in correlating this information with available data on the percentage of their other constituents.

The percentage of methoxyl in the plants and plant materials used was also determined. The methoxyl group, which is widely distributed in the plant kingdom, is found in many naturally occurring organic compounds, in alkaloids, in various constituents of volatile oils, and in all lignified plant materials, such as straw, hulls, cobs, stalks, leaves, trunks of trees, and shrubs. Owing to the scarcity of information on the methoxyl content of plants in general and of food plants in particular, it seemed of interest to obtain additional knowledge.

The method employed for the estimation of uronic acids is fairly selective and determines a definite and specific class of organic compounds, whereas some of the empirical methods used for plant analysis determine classes of compounds wholly unrelated chemically.

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[1] Contribution No. 224 from the Color and Farm Waste Division.

In the case of the methoxyl group the method is based on the reaction between ethers and hydriodic acid. A full discussion of the principle involved, a review of the literature, and a method for determination of methoxyl, have been described in a recent communication by Phillips.[1] This determination is also specific although it determines the alkoxyl rather than the methoxyl group, nevertheless alkoxyl groups higher than ethoxyl practically never occur in plant materials and ethoxyl groups are found only rarely. It is therefore safe to say that the alkyl iodide obtained is practically entirely methyl iodide and that the method actually determines methoxyl groups.

All the methods[2] described in the literature for the determination of uronic acids are based on a reaction which was first carefully studied by Léfevre and Tollens[3] and which formed the basis of their method for the quantitative estimation of uronic acids. It is based on the fact that when a uronic acid, such as glucuronic or galacturonic, is heated with 12 per cent hydrochloric acid, as in the determination of pentosans, it is decomposed into furfural, carbon dioxide, and water, as indicated by the following equation:

$$\begin{array}{l} \text{H-C}=\text{O} \\ \text{H-C-OH} \\ \text{H O-CH} \\ \text{H O-CH} \\ \text{H-C-OH} \\ \quad\text{COOH} \end{array} \qquad \begin{array}{l} \text{H C}\text{---}\text{C H} \\ \quad\|\qquad\| \\ \text{H C}\qquad\text{C-C}=\text{O}+\text{CO}_2+3\text{H}_2\text{O}. \\ \quad\diagdown\quad\diagup\ \text{H} \\ \qquad\text{O} \end{array}$$
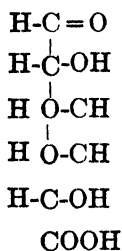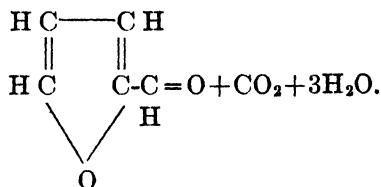
d-Galacturonic Acid   Furfural

The quantity of furfural given off is less than that required by the equation above, whereas the yield of carbon dioxide is quantitative. Accordingly, the method for the determination of uronic acids resolves itself into a determination of the quantity of carbon dioxide given off when a given weight of a plant substance is heated with 12 per cent hydrochloric acid.

It will be observed from the equation given that one mole of uronic acid yields one mole of carbon dioxide, and as the molecular weight of the inner anhydride or lactone of glucuronic or galacturonic acid is 176 and that of carbon dioxide 44, it follows therefore that the percentage of carbon dioxide obtained multiplied by 4 gives the percentage of uronic acid anhydride in the sample.

EXPERIMENTAL

Preparation of Plant Material for Analysis.—The fresh plant material was first dried in a steam-heated drier provided with an air blower and maintained at a

[1] *This Journal,* 15, 118 (1932).
[2] *J. Soc. Chem. Ind.,* 44, 253 T (1925); *J. Am. Chem. Soc.,* 48, 232 (1926); 52, 775 (1930); 53, 1999 (1931); *Analyst,* 57, 220 (1932).
[3] *Ber.,* 40, 4513 (1907).

temperature of 70 to 90° C. The partly desiccated material was then ground in a mill, dried in an oven at 105° C., and preserved in well-stoppered containers. When honey-dew melon was subjected to the temperature indicated, considerable darkening took place. The material was accordingly dried in vacuo over sulfuric acid in the Abderhalden drier at 56° C.

DETERMINATION OF URONIC ACIDS

APPARATUS

The apparatus described by Dickson, Otterson and Link[1] was used in all analyses and was found to be entirely satisfactory.

PROCEDURE

The analytical procedure followed was a modification of the Dickson, Otterson and Link method made by the writers in order to eliminate any possible error in the results in the event that the plant material contained carbonates. The following is a detailed description of the method used.

The weighed sample (1–2 grams) was placed in the reaction flask, and 100 cc. of 12 per cent hydrochloric acid and a few chips of unglazed porcelain were added. The contents of the reaction flask were heated to 70° C. and maintained at this temperature for one-half hour to decompose carbonates, should any be present. (The uronic acids do not give off their carbon dioxide at this temperature.) The reaction flask was then connected to the condenser and the other part of the apparatus, and a current of air free from carbon dioxide was swept through for 20 minutes in order to remove any carbon dioxide. The contents of the reaction flask were heated in an oil bath, and as soon as boiling began a definite volume of 0.2 N barium hydroxide was run into the absorption tower. Sufficient carbon dioxide-free water to cover all the glass beads was then added to the tower. The temperature of the oil bath was maintained at 135–140°C. for 5 hours while a small stream of carbon dioxide-free air was passed through the system. At the end of the 5-hour period, the absorption tower was disconnected and washed down into the flask with carbon dioxide-free distilled water. The unused barium hydroxide was then determined by titration against 0.1 N hydrochloric acid, phenolphthalein being used as the indicator. A blank determination was then carried out exactly as described, except, of course, no sample was added to the reaction flask.

It was found that in the blank determination 0.15 cc. of 0.2 N barium hydroxide was used. The number of cubic centimeters of 0.2 N barium hydroxide used (minus that required for the blank) multiplied by 0.0044 and 100 and divided by the weight of the sample taken gave the percentage of carbon dioxide given off by the sample. The percentage of carbon dioxide multiplied by 4 gave the percentage of uronic acid anhydride in the sample.

DETERMINATION OF METHOXYL

The percentage of methoxyl in the plant material was determined by the method described in the previous paper.[2]

The results obtained are recorded in Table 1.

A marked difference in the percentage of uronic acids found in the several plant materials examined is shown. It may also be noted that the uronic acids are present in substantial amounts, not as mere traces. Because uronic acids are known to occur in pectin material, a greater per-

[1] *J. Am. Chem. Soc.*, **52**, 775 (1930).
[2] *Loc. cit.*

*Results of determination of uronic acid anhydride and methoxyl in certain
plant substances*
(Unless stated otherwise, analyses were made on materials dried at 105°C.)

PLANT SUBSTANCE	CO_2	URONIC ACID ANHYDRIDE	OCH_3[a]
	per cent	*per cent*	*per cent*
Honey Dew Melon[†]	0.90	3.60	0.15
Cantaloupe	1.00	4.00	0.23
Lima Beans	1.05	4.20	0.86
Peas	1.22	4.88	0.74
Peeled Cucumbers	2.08	8.32	0.94
Asparagus Stalks	2.29	9.16	1.48
Asparagus Tips	2.47	9.88	1.36
Carrots	2.56	10.24	0.64
Spinach	2.58	10.32	0.96
Summer Squash	2.66	10.64	0.89
Cabbage Leaves	2.79	11.16	0.97
Pea Pods	2.83	11.32	2.06
Cucumber Peelings	2.99	11.96	0.85
Cauliflower	3.14	12.56	0.93
Radish Tops	3.18	12.72	0.80
Egg Plant	3.27	13.08	1.07
Apple Peelings	3.29	13.16	1.53
Kale	3.51	14.04	0.82
Head Lettuce Leaves	3.55	14.20	0.76
Beet Tops	3.63	14.52	0.66
Carrot Tops	4.07	16.28	1.50
Celery (Leaves and Stalks)	4.18	16.72	0.80
Orange Peelings	4.43	17.72	2.32

[a] Results are the averages of at least two determinations concordant within the limits of experimental error.
[a] Determinations made on material extracted with alcohol-benzene solution and results calculated on the basis of unextracted material dried at 105° C.
[†] Dried in vacuo in Abderhalden drier over sulfuric acid.

centage of uronic acid is to be expected in plant materials that are rich in pectin. The results do not indicate any correlation between the percentage of uronic acid anhydride and the methoxyl content of the plant materials examined.

CORRECTION

Vol. XVI, p. 83—Phenolsulfonates.

For the first two lines substitute the following: "Dissolve the sample (equivalent to about 0.8 g of phenolsulfonates) in about 30 cc of H_2O and add 5 cc of HCl. Titrate with 0.4 N Br (11.134 g of $KBrO_3$+50 g of KBr diluted to 1 liter with H_2O and standardize 10 cc portions against 0.1 N $Na_2S_2O_3$)."

REPORT ON EGGS AND EGG PRODUCTS

By Samuel Alfend[1] (U. S. Food and Drug Admin-
istration, St. Louis, Mo.), *Referee*

The Association recommended eleven topics for study in 1932. The methods for glycerol and unsaponifiable matter were assigned to the referee.

GLYCEROL

The qualitative test for glycerol described in last year's report[2] was studied by three collaborators. N. L. Knight reported a strong positive test for a sample of egg yolk containing 2 per cent of glycerol and a negative test for egg yolk containing 10 per cent added sucrose but no added glycerol. L. Jones, Kansas City Food and Drug Laboratory, reported as follows:

Approximately 5 grams of egg yolk—0.5 gram of sucrose. Slight pink color developed at the end of 2 minutes and quite a strong pink at the end of 5 minutes.

Approximately 5 grams of egg yolk—4 drops of glycerine. A perceptible pink color developed in about 30 seconds. Strong violet color within 5 minutes.

L. C. Mitchell obtained a strong positive test on the sample containing glycerol, and a slight pink color within 1 minute on the yolk containing only added sugars.

Based on these results, the wording of the last sentence of the method should be changed to read: "In the presence of acrolein (due to glycerol) a strong pink color develops within 1 minute, and becomes a deep violet-red within 5 minutes."

The method of de Coquet,[3] in which glycerol is oxidized to dihydroxy-acetone, and a blue green color is developed in the presence of codeine and sulfuric acid, is an excellent test for glycerol when no other organic matter is present, but the detailed purification necessary to make it applicable for eggs was found to preclude its use as a rapid qualitative test in eggs.

The quantitative method described in last year's report was subjected to collaborative study. The results (see Table 1) were somewhat disappointing as regards the blank on the egg. The blank obtained by the referee did not exceed 0.1 per cent, but the collaborators reported blanks from 0.21 to 0.92 per cent. The recovery of added glycerol was for the most part sufficiently good for the purpose in view. The collaborators experienced difficulty in recovering 75 cc. of solution after the magnesium carbonate filtration. An aliquot of 50 cc. should therefore be used in the future. Further work to determine the cause of the high blank is desirable.

[1] Presented by J. O. Clarke.
[2] *This Journal*, 15, 331 (1932).
[3] *Chem. Ind.*, 23, 1509 (1930); *C.A.*, 24, 4580 (1930).

TABLE 1

Collaborative results for glycerol in egg.

ANALYST	BLANK	GLYCEROL		AVERAGE RECOVERY
		ADDED	RECOVERED	
	per cent	*per cent*	*per cent*	*per cent*
Jones	0.21	8.86	9.09	97
		7.82	7.07	
Buell	0.82	7.95	7.70	97
	0.92	7.95	7.73	
Pruitt	0.51	10.67	10.72*	98
	0.49	9.61	10.04*	
Haenni	0.37	9.09	9.08*	97
	0.34	9.64	9.74*	
Average	0.46	8.95	8.90	97

* Total, including blank.

Continuing his efforts to devise a quantitative method satisfactory in the presence of added sugars, the referee attempted to separate out the glycerol by co-precipitation with zirconium hydroxide. Griessbach, Bitterfeld and Weiss[1] claim a 90 per cent recovery on a commercial scale from 10 per cent solutions of glycerine. Following their directions for a hot precipitation, the referee obtained yields of 75–79 per cent of aqueous solutions of glycerol, the remainder of the glycerol being recovered in the filtrate, as shown by dichromate oxidation. Two determinations involving cold precipitation yielded close to theoretical results (100–101 per cent). The method therefore presents some promise, and should be studied further.

UNSAPONIFIABLE MATTER

The crude lipoids obtained by extraction of egg with chloroform-alcohol solvent have given more consistent results for unsaponifiable matter than those found on the lipoids extracted by alcohol and ether. This was true for both the modified Kerr-Sorber and the F. A. C. procedure. It is not yet apparent which of the two saponification procedures will yield the best results.

No opportunity was presented for work on the sterol content of the unsaponifiable matter. The three most promising procedures for investigation seem to be: (1) Lampert's adaptation[2] of the colorimetric Liebermann-Burchard reaction; (2) the digitonin method of Tillmans, Riffart and Kuhn;[3] and (3) Perlman's gravimetric modification[4] of (2).

[1] German Patent No. 501110. June 27 (1930).
[2] *Ind. Eng. Chem.*, Anal. Ed., **2**, 159 (1930).
[3] *Z. Untersuch. Lebensm.*, **60**, 361 (1930).
[4] Private communication.

The two latter methods have the drawback of requiring the use of an expensive reagent.

TOTAL PHOSPHORUS

Associate Referee L. C. Mitchell reported excellent results for total phosphoric acid (P_2O_5) in this year's collaborative work. His report includes a detailed description of his preliminary work in 1930. The referee approves of his recommendation that the tentative method be adopted as official, and that the heading be changed from "Phosphoric Pentoxide" to "Phosphorus."

FAT (BY ACID HYDROLYSIS)

Mitchell's report presents data indicating that hydrolysis at 75°–80°C. does not completely hydrolyze the lecithin, and contains collaborative results on the method providing for hydrolysis at the temperature of boiling water. He recommends that the method as modified for this year's collaborative work be adopted as tentative, and that further collaborative work be performed. This is approved.

LIPOIDS AND LIPOID PHOSPHORIC ACID (P_2O_5)

On the basis of this year's collaborative work, the associate referee recommends the adoption of the modification of the chloroform-alcohol extraction as tentative, to replace the present tentative method. The suggested procedure minimizes the concentration error by eliminating the filtration of the first extract, but does not provide a correction for the volume of insoluble solids. This will involve an error of roughly 0.5 per cent for dried yolk, 0.4 per cent for dried whole egg, 0.25 per cent for liquid yolk, and 0.05 per cent for liquid whole egg.

$$X = \frac{100\,(V-v)\,1}{Wa}, \text{ in which}$$

X = true percentage of lipoids,
l = wt. of lipoids found in aliquot,
a = volume of aliquot,
V = total volume to which sample is made up, and
v = volume of insoluble solids in sample.

The referee approves the recommendation of the associate referee, provided the correction for the volume of insoluble solids is incorporated in the method.

The method for lipoid phosphoric acid has been altered in some details. The alterations are apparently based on sound principles, and the referee approves of the method as now offered for tentative adoption, with the exception that the needless specification for "collecting the filtrate in a 300 or 500 cc. Erlenmeyer flask" should be eliminated. There is no point

in specifying the kind of vessel in which a phospho-molybdate precipitation should be carried out.

REDUCING SUGARS AND SUCROSE

Mitchell has submitted satisfactory collaborative results on reducing sugars in eggs. The recovery of added sucrose was remarkably close to theoretical. The referee calls attention here to one detail—the inversion with 5 cc. strong hydrochloric acid. Mitchell specifies the method given in *Methods of Analysis*, A.O.A.C., 1920, (p. 75, 14), except that his temperature and time specifications are indefinite. The two subsequent editions (1925), p. 187, 23(c) and 1930, p. 373, 23(c) specify 10 cc. of acid of sp. gr. 1.1029, with precautions as to time of inversion and temperature. It appears desirable to conform with the general method for sugars unless some specific advantage inheres in a change. The associate referee recommends the method for adoption as official.

Mitchell has pointed out that the error caused by the volume of insoluble matter may amount to 7.5 per cent in the case of liquid yolk. In the case of yolks containing large amounts of added sugar this error will be important. It is therefore recommended that the method as adopted specify a correction for the volume of the insoluble portion of the sample.

WATER-SOLUBLE AND CRUDE ALBUMIN NITROGEN

The associate referee recommends the adoption as official of the tentative methods for water-soluble nitrogen and water-soluble nitrogen precipitable by 40 per cent alcohol in liquid eggs,[1] on the basis of this year's collaborative work. The results for dried eggs were not satisfactory, and the associate referee recommends further study of the method as it applies to dried eggs. The referee concurs in these recommendations.

CHLORINE

The excellent collaborative results on the tentative method for chlorine[2] warrant the associate referee's recommendation that the method be adopted as official.

No report was submitted on detection of decomposition.

RECOMMENDATIONS[3]

It is recommended—

(1) That the tentative method for the determination of total phosphoric acid (P_2O_5) be adopted as official (first action).

(2) That the method for the determination of fat (by acid hydrolysis) described in the associate referee's report be adopted as tentative to replace the present tentative method.

[1] *This Journal*, 15, 75 (1932).
[2] *Methods of Analysis*, A.O.A.C., 1930, 249.
[3] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 55 (1933).

(3) That the method described in the associate referee's report as the "extraction modification," be amended by adding at the end "Correct for the volume of the insoluble solids. (When the volume is unknown, the correction is roughly, 0.5 per cent for dried yolk, 0.4 per cent for dried whole egg, 0.25 per cent for liquid yolk, and 0.5 per cent for liquid whole egg.)," and that the method be adopted as tentative to replace the present tentative method.

(4) That the method for determination of reducing sugars and sucrose[1] be adopted as tentative after the following sentence has been added before the last sentence in (h) and also in (i): "Correct for the volume of insoluble sample."

(5) That the study of the methods for unsaponifiable matter and its constituents be continued.

(6) That the tentative methods for the determination of water-soluble nitrogen and water-soluble nitrogen precipitable by 40 per cent alcohol, as applied to liquid eggs, be adopted as official (first action), and that the tentative methods for dried eggs be further studied.

(7) That work to perfect the method for acid-soluble phosphoric acid (P_2O_5) be continued.

(8) That the tentative method for the determination of chlorine be adopted as official (first action).

(9) That the qualitative test for glycerol described in the 1931 report of the referee be amended by changing the last sentence to read: "In the presence of glycerol (due to acrolein) a strong pink color develops within 1 minute and becomes a deep violet-red within 5 minutes,"—that it be adopted as tentative, and that it be further studied with a view to adoption as official.

(10) That the quantitative method for glycerol described in the 1931 report of the referee[2] be adopted as tentative, with the heading, "Glycerol (not applicable in presence of added sugars)"; that further studies be made to determine the cause of the high blank; and that further studies on the precipitation method described in this report be made, to be followed by collaborative work.

(11) That methods for determining ammonia nitrogen as an index of decomposition in liquid egg be studied.

(12) That the rapid method for acidity of ether extract described in the associate referee's report for 1931[3] be studied further with a view to making it applicable to both liquid and dried eggs.

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[1] *This Journal*, 14, 397 (1931).
[2] *Ibid.*, 15, 334 (1932).
[3] *Ibid.*, 341.

REPORT ON TOTAL PHOSPHORUS (P_2O_5), FAT (BY ACID HYDROLYSIS), LIPOIDS AND LIPOID PHOSPHORUS (P_2O_5), REDUCING SUGARS (DEXTROSE), WATER-SOLUBLE AND CRUDE ALBUMIN NITROGEN, AND CHLORINE IN EGGS

By LLOYD C. MITCHELL[1] (U. S. Food and Drug Administration, St. Louis, Mo.), *Associate Referee*

This report includes the previous unpublished work of the associate referee on the development of the methods, and the collaborative results obtained this year.

TOTAL PHOSPHORUS (P_2O_5)

Until 1930 the Association had made no study of total phosphorus in eggs. The 1925 edition of *Methods of Analysis* provided several different procedures, varying somewhat with the product under analysis, for the preparation of the solution used in the phosphorus determination. To prepare the solution on tea, coffee, vinegar, and gelatine, the ash is dissolved in nitric or hydrochloric acid, and is then usually boiled; on baking powder, beer, wine, and plant material, the ash obtained by ignition after the addition of magnesium nitrate or calcium acetate or sodium carbonate is dissolved in nitric or hydrochloric acid, with or without subsequent boiling; on fertilizers, the organic matter is destroyed by ignition directly or after the addition of magnesium nitrate, and the resulting ash is dissolved in strong acid, or by one of the several wet digestion methods; on mineral waters, the water is evaporated to dryness with nitric acid; on meats, the organic matter is destroyed by wet digestion; and on soils, the ash obtained by ignition with sodium peroxide or ammonium chloride and calcium carbonate is dissolved in acid.

One sample of yolks dried with magnesium nitrate, ignited, and the resulting ash dissolved by boiling in dilute nitric acid (1+3) yielded 1.28 and 1.15 per cent phosphorus (P_2O_5). The discrepancy in the results is probably due to the loss of material caused by splattering during the unusually rapid combustion.

Three methods were used to prepare solutions for the phosphorus determination on whites and yolks separated from a sample of eggs. Method 1: When boiled in dilute nitric acid (1+3) the ash obtained by ignition gave 0.04 and 0.04 per cent phosphorus (P_2O_5) for whites and 1.10 and 1.06 per cent for yolks. Method 2: When boiled in dilute nitric acid (1+3) the ash obtained by ignition with magnesium acetate yielded 0.04 and 0.04 per cent phosphorus (P_2O_5) for whites and 1.26 and 1.27 per cent for yolks. Method 3: by wet digestion with strong nitric and

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hydrochloric acids, the ash showed 0.03 and 0.04 per cent phosphorus for whites and 1.09 and 1.14 per cent for yolks.

On the whites and yolks separated from another sample of eggs, a fourth method instead of Method 1 was used to prepare the solution. Method 4 consisted in drying the egg material after addition of sodium carbonate solution, charring the dried residue for 1 hour at 500°, and leaching with cold dilute nitric acid (1+3). Method 2 gave 0.03 and 0.04 per cent phosphorus (P_2O_5) for whites and 1.34 and 1.39 per cent for yolks: Method 3, 0.04 and 0.04 per cent for whites and 1.30 and 1.30 per cent for yolks; and Method 4, 0.04 and 0.04 per cent for whites and 1.36 and 1.37 per cent for yolks.

Since charring the egg material with sodium carbonate for 1 hour at 500° to prepare the solution for the phosphorus determination appeared satisfactory, in order to check the recovering of phosphorus under these conditions, the following experiment was made: Sodium phosphate was heated with sodium carbonate in the presence of organic matter (sucrose and olive oil) in Pyrex beakers at 400°, 500°, 600°, 700°, and 800° for 1 hour and the charge was made slightly acid with dilute nitric acid (1+3), filtered, and washed. A parallel experiment with alcoholic potash instead of sodium carbonate was also made. The amount of sodium phosphate, as P_2O_5, used was 21.2 and 21.3 mg. The blanks on the organic matter (sucrose and olive oil) were nil. At 800° the beakers softened at the end of 15 minutes, and collapsed at the end of 30 minutes. The slightly amber colored filtrates on the samples heated at 400° indicate incomplete combustion of the organic matter and probably account for the somewhat low results for phosphorus. The recovery of phosphorus (P_2O_5) is shown in Table 1.

TABLE 1

Recovery of sodium phosphate as P_2O_5 when ignited at various temperatures with sodium carbonate and alcoholic potash in presence of organic matter.

TEMPERATURE OF IGNITION	IGNITED WITH—	
	SODIUM CARBONATE	ALCOHOLIC POTASH
°C.	mg.	mg.
400	19.9	19.5
	20.9	20.0
500	21.5	21.2
	21.2	21.2
600	21.5	21.3
	21.6	21.5
700	21.3	21.4
	21.3	21.2

It was observed (1) that alcoholic potash retards the combustion of carbon to a much greater degree than does sodium carbonate; (2) that alcoholic potash attacks the glass considerably more and at lower temperatures than does sodium carbonate; (3) that the charge need not be free from carbon provided a colorless solution is obtained. (On egg materials, repeated tests for both phosphorus and chlorine, after the washed charred residue has been ignited to an ash, have been negative); (4) that excess alkali prevents any volatilization of the phosphorus when subjected to temperatures not exceeding 700; (5) that excess of the alkali cation assures formation of tertiary phosphates during the charring process, hence heating or boiling the charge with acid is not necessary; (6) that apparently alcoholic sodium hydroxide would be preferable to alcoholic potassium hydroxide as a fixative for phosphorus (see method for lipoid phosphorus); and (7) that the Pyrex beakers used withstood a muffle temperature of nearly 800° before softening and collapsing.

The sodium carbonate method of preparing the solution for the phosphorus determination was studied collaboratively in 1930 and reported by Associate Referee Bornmann.[1]

For this year's collaborative results see Tables 6, 7, and 8.

FAT (BY ACID HYDROLYSIS)

The tentative method[2] was modified to provide that the sample be hydrolyzed in a Mojonnier fat extraction tube instead of in a beaker, thus eliminating the possibility of loss of fat in transferring the hydrolyzed sample to an extraction tube. Hitchcock[3] studied the method collaboratively on liquid eggs, and recommended that it be adopted as a tentative method. Four analysts reported on the sample, and their results showed a maximum variation of 0.57 per cent. Palmer[4] failed to obtain concordant results when applying the method to dried whole eggs. His experimental data indicate that a digestion temperature of 75°–80° gives a maximum yield. Hertwig,[5] in recommending that the method be adopted as a tentative method, states that the experimental error is high for dried eggs.

As preliminary tests, for example, Yolk 1 yielded 30.79 and 30.67 per cent fat, Yolk 2, 30.74 and 30.84 per cent, and Yolk 3 (separated from eggs after cooking by dried heat), 29.43 and 29.51 per cent (63.54, 63.30; 63.53, 63.66; and 62.84, 63.01 per cent, respectively, in the dry substance), revealed no difficulty in obtaining satisfactory checks, the tentative method, as above modified, was submitted for collaborative study in 1930, and the results were reported by Associate Referee Bornmann.[1] The agreement among the collaborators was not considered to be entirely satisfactory.

[1] *This Journal*, 14, 416 (1931).
[2] *Methods of Analysis*, A.O.A.C., 1930, 246.
[3] *This Journal*, 8, 610 (1925).
[4] *Ibid.*, 615.
[5] *Ibid.*, 596.

A sample of whole eggs, divided into three portions and analyzed on successive days, yielded 10.86 and 10.83, 10.47 and 10.43, and 10.37 and 10.56 per cent fat, respectively, The comparatively wide variation in results on the separate subdivisions examined at different times indicated the possibility of incomplete action of the acid on the fatty substances of the egg. The fatty residues were found to contain varying quantities, from 0.2 to 0.4 per cent, of phosphorus (P_2O_5). (The egg lipoids, which, calculated from the nitrogen and phosphorus contents, consist of approximately one-third lecithin, contain about 2.78 per cent phosphorus (P_2O_5).) Another determination on the portion which gave 10.86 and 10.83 per cent fat, showed 10.55 per cent when heated at 70°, and yielded an additional amount of 0.42 per cent when heated further in a boiling water bath for 20–30 minutes.

Repetition of the fat determinations, on approximately 2.3 and 4.6 grams of sample, respectively, for the duplicates, and hydrolyzation by inserting the Mojonnier tubes into boiling water bath for 20 minutes, yielded 11.12 and 11.09, 11.02, and 11.07, and 11.04 and 11.14 per cent fat, respectively, in the three subdivisions. The fatty residues extracted from 2.3 gram portions of sample gave negative tests for phosphorus, and from 4.6 gram portions, a trace of the yellow precipitate.

A sample of spray-dried yolks showed, on hydrolyzing in a boiling water bath for 20 minutes, 60.06 and 60.08 per cent fat, which was practically free from phosphorus. On subsequent examinations, the sample of dried yolks yielded 60.02, 60.13, 60.00, 59.98, 60.02, and 60.02 per cent fat.

On dairy products, with the exception of cheese, the official methods[1] provide for the addition of alcohol just previous to the extraction of the fat with ether and petroleum ether. On cereal products and egg products, the tentative methods also make this provision in the fat extraction. The use of water instead of alcohol to fill the lower bulb of the extraction tube was found to shorten materially the length of time necessary for the ether layer to become clear. The use of twice as much petroleum ether as ether decreases the time necessary for the ether layer to clear, but materially increases the time necessary to evaporate the ethers. The results obtained by addition of either alcohol or water to fill the lower bulb checked closely.

During his 1932 work on commercially separated eggs Alfend (private communication) submitted the comparative results given in Table 2 on the use of water, or alcohol, to fill the lower bulb of the extraction tube in the fat determination.

He commented: "With whole eggs, samples to which water had been added tended to give troublesome emulsions which were usually helped

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[1] *Methods of Analysis*, A.O.A.C., 1930, 217, 225, 227, 228, 230, 243.

TABLE 2

Comparative results for fat (by acid hydrolysis) on commercially separated eggs; water, or alcohol, used to fill lower bulb of extraction tube.

| SUBSTANCE | FAT, BY ACID HYDROLYSIS | |
	ALCOHOL	WATER
	per cent	per cent
Whole egg	11.74	11.69
Whole egg	12.89	12.93
Whole egg	12.11	12.11
Yolk	29.10	29.03
Yolk	25.03	24.93
Yolk	28.84	28.88

by the addition of 0.1–1.0 cc. alcohol. With yolks, samples to which water had been added cleared more quickly and completely."

A reasonable variation in the weight of sample used in the fat determination appears to have no effect upon the percentage of fat obtained. Portions of commercially separated yolks weighing approximately 1.2, 1.8, and 3.4 grams yielded 25.19, 24.96, and 25.07 per cent fat; while portions of commercially separated whole eggs weighing 1.3, 1.8, and 3.7 grams gave 12.78, 12.78, and 12.85 per cent fat, respectively.

For this year's collaborative results, see Tables 6, 7, and 8.

LIPOIDS AND LIPOID PHOSPHORUS (P_2O_5)

The tentative method for the determination of lipoids and lipoid phosphorus (P_2O_5)[1] was found by Hertwig[2] to yield markedly higher results than are obtained by other available methods, as Juckenack's method[3] and Rask and Phelps' method (unpublished).

A mixture of equal parts of absolute alcohol and chloroform was found

Amount and analysis of lipoids obtained by two methods.

| SUBSTANCE | TENTATIVE METHOD | | | ALCOHOL-CHLOROFORM METHOD | | |
	LIPOIDS	P_2O_5*	NITROGEN*	LIPOIDS	P_2O_5*	NITROGEN*
	per cent	per cent	per cent	per cent	per cent	per cent
Whites	0.04	Trace	—	0.04	Trace	—
	0.04		—	0.06		—
Yolks	31.17	2.76	—	33.21	2.83	—
	31.58	2.72	—	33.26	2.86	—
Whole Eggs	12.83	—	0.64	13.46	—	0.69
	12.89	2.83	—	13.41	2.90	—

* On basis of the lipoid.

[1] *This Journal*, 8, 273 (1925).
[2] *Ibid.*, 7, 91 (1923).
[3] *Z. Nahr. Genussm.* 3, 1 (1900).

to be an excellent solvent for lipoids of eggs. As 100 cc. of the mixed solvent absorbs about 13 cc. of water, the solvent lends itself to the direct extraction of the lipoids from liquid eggs without any preliminary treatment.

The alcohol-chloroform method[1] gave somewhat higher results for lipoids than did the tentative method, but the composition of the lipoids obtained by both methods appears similar, as shown in the preceding table.

The results obtained for lipoid phosphorus (P_2O_5) during the 1930 collaborative work[2] showed that something happened to the phosphorus. A brief chemical study[3] indicated that the low results for lipoid phosphorus (P_2O_5) was due to a bacterial destruction of the lecithin.

For this year's collaborative study, some changes were made in the method. Since the work on the phosphorus determination recorded in this report indicated that the sodium cation was preferable to the potassium cation as a fixative for the phosphorus, alcoholic sodium hydroxide solution was substituted for the alcoholic potash solution. Glass beakers were used instead of platinum dishes for weighing the lipoids, and later they were charred in preparing the solution for the phosphorus determination. A washing modification of the method was included in the directions to the collaborators with a view to ascertaining the error due to the volume of the insoluble egg material and to a possible evaporation during filtration. The modifications of the method as submitted to the collaborators are given later in this report.

With a view to eliminating any error in the preparation of the solution which may be due to the probable evaporation of the solvent during filtration in the extraction modification of the method, some results were obtained for lipoids and lipoid phosphorus (P_2O_5) by filtering and pipetting in a closed system, and by pipetting without filtering. Some results were also obtained on the washing modification of the method. The results reported herein were obtained on yolks separated from commercial fresh eggs. A partial history of these eggs and of the composition of the yolks separated therefrom has been published.[4]

The details of the modifications of the method used in the preparation of the solution follow:

PREPARATION OF SOLUTION

(c) Weigh accurately, by difference, approximately 4 grams of the well-mixed sample into a 100 cc. volumetric flask; add slowly (dropwise from a pipet), with constant mixing until the proteins become coagulated and then thoroughly broken up, 25 cc. of the mixed solvent; and allow the mixture to stand 1 hour, mixing at 5 minute intervals.

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[1] *This Journal*, 14, 418 (1931).
[2] *Ibid.*, 422.
[3] *Ibid.*, 15, 282 (1932).
[4] *Ibid.*, 310.

(c-1) *Filter and pipet in a closed system, using the T-tube gadget.*—Fill to the mark with the solvent and shake. Pass a 50 cc. pipet through a T-tube, making joints tight with thin rubber tubing; wrap a filter paper around tip of pipet, tying it tightly to pipet with a linen thread; insert the pipet into the flask and fill by applying gentle pressure by means of a rubber bulb attached to the T-tube; and transfer a 50 cc. aliquot to a 250 cc. beaker.

(c-2) *Pipet without filtering.*—Fill to mark with the solvent, shake, allow to stand until clear, and transfer a 50 cc. aliquot to a 250 cc. beaker.

(c-3) Decant through a 7 cm. filter paper into a 250 cc. beaker. Rinse flask and wash residue and filter paper thoroughly with 5 cc. portions of the mixed solvent, breaking up the coagulated material with a stirring rod. Continue the washings with 5 cc. portions of the mixed solvent, the breaking up of the coagulated material with a stirring rod, and the washing of the edges of the filter paper until the lipoids are removed.

The results found are given in Table 3.

TABLE 3

Lipoids and lipoid phosphorus in yolks separated from commercial fresh eggs.

SAMPLE NUMBER	METHOD MODIFICATION	IN ORIGINAL SUBSTANCE		IN DRY SUBSTANCE		RATIO
		LIPOIDS				
		TOTAL	P₂O₅	TOTAL	P₂O₅	$\dfrac{P_2O_5}{LIPOIDS}$
		per cent	per cent	per cent	per cent	per cent
25	c-1	35.10	0.98	69.50	1.94	2.79
26	"	35.43	0.96	69.59	1.89	2.71
27	"	35.57	0.97	69.90	1.91	2.73
28	"	35.12	0.98	69.46	1.94	2.79
29	"	35.23	0.99	69.32	1.95	2.81
30	"	34.98	1.01	69.38	2.00	2.89
31	"	35.03	1.01	69.41	2.00	2.88
32	"	35.34	0.98	69.55	1.93	2.77
33	"	34.96	0.97	69.32	1.93	2.77
34	"	35.17	0.97	69.14	1.91	2.76
35	"	34.71	0.95	69.85	1.91	2.74
37	c-2	35.32	0.98	68.81	1.91	2.77
38	"	35.44	1.00	69.68	1.97	2.82
39	"	36.20	0.98	70.54	1.91	2.71
40	"	35.43	0.99	69.46	1.94	2.79
41	c-3	34.48	0.96	68.88	1.92	2.78
42	"	34.33	0.97	68.98	1.95	2.83
43	"	34.63	0.97	69.18	1.94	2.80
44	"	34.41	0.97	69.22	1.95	2.82
45	"	35.01	0.99	69.14	1.95	2.83
46	"	35.03	0.95	69.70	1.89	2.71
Maximum		36.20	1.01	70.54	2.00	2.89
Minimum		34.33	0.95	68.81	1.89	2.71
Average		35.09	0.98	69.43	1.94	2.79

For this year's collaborative results, see Tables 6 and 8.

REDUCING SUGARS (DEXTROSE)

The method used by Redfield and his co-workers[1] depends on heat and a small amount of acetic acid for clarification before the determination of the reducing substances. When the egg material contains sugar, heating in the presence of acetic acid, however, causes a partial inversion of the sucrose. A sample of yolks containing 3.7 per cent of added sucrose showed 1.3 per cent of reducing substances direct, as invert sugar, determined immediately after clarification with heat and acid.

Clarification with the mineral salts usually employed in reducing sugars determinations, such as neutral and basic lead acetate, copper sulfate, mercuric nitrate, phosphotungstic acid, proved unsatisfactory. The addition of a small amount of acid (5 cc. of 0.01 N acid per gram of egg material) at room temperature yields a clear filtrate, but does not remove the water-soluble substances, such as albumin. The addition of acid, even though in small quantities, may cause some inversion of sucrose when present. Fifty per cent alcohol in the presence of a small amount of sodium chloride precipitates the albumin. This strength of alcohol prevents enzymic action.[2] The 50 per cent alcohol and the small amount of salt, however, do not remove all the interfering water-soluble nitrogenous substances derived from the yolk. These substances are removed by phosphotungstic acid. Based on the above noted observations, a method for the determination of the reducing substances, before and after inversion, was worked out.[3]

Table 4 gives the results obtained on various egg materials with and without added sucrose.

TABLE 4

Results on egg material with and without added sucrose.

REDUCING SUGARS	WITHOUT SUCROSE			WITH SUCROSE		
	WHOLE	WHITE	YOLK	WHOLE	WHITE	YOLK
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Direct as dextrose	0.35	0.35	0.15	0.42	0.39	0.27
Invert as dextrose	0.35	0.35	0.18	10.03	10.03	9.85
Redfield method as dextrose	0.33	0.30	0.18	---	—	—
Sucrose recovered*	—	—	—	102.5	101.5	107.0

* No correction made for volume occupied by precipitate, largely proteins, or proteins and fat.

The collaborative results for 1930 on the first sample of whole eggs

[1] U. S. Dept. Agr. Bull. 846, p. 93 (1930); *This Journal*, 6, 8 (1922).
[2] U. S. Dept. Agr. Bur. Chem. Cir. 71.
[3] *This Journal*, 14, 397 (1931).

submitted to the collaborators[1] showed 0.33, 0.32, 0.30, 0.31, 0.29, and 0.29 per cent dextrose; and on the second sample (results received too late for the referee to include in his 1930 report), 0.34, 0.11, 0.30, nil, nil, trace, nil, 0.33, 0.01, 0.33, and 0.29 per cent dextrose. On the second sample collaborator Macomber commented as follows: "I am reporting no dextrose * * *. The eggs appeared to be fresh and should have contained about 0.30 per cent dextrose. If this result is in error it must be due to allowing the material to stand for several days. After I had * * * added the alcohol * * * it stood in that condition for a week * * * before the determination was completed."

The variation in the collaborative results on the second sample is undoubtedly due to the decomposition of the dextrose before the determinations were started as the writer has allowed samples, after the addition of alcohol, to stand over a month without loss of reducing substances. When liquid egg material is allowed to stand at room temperature, the reducing substances gradually disappear. Usually the change, or decomposition which may be complete, cannot be detected organoleptically, although the whole egg material appears to be slightly less alkaline. On the second collaborative sample, the writer reported 0.34 per cent dextrose on the first day, 0.32 per cent on the second day, and 0.26 per cent on the third day after preparation of the sample.

For this year's collaborative results see Tables 6 and 8.

WATER-SOLUBLE NITROGEN AND WATER-SOLUBLE NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL (CRUDE ALBUMIN NITROGEN)

The work on the development of the method and the results of previous collaborative study were reported at the 1931 meeting.[2]

For this year's collaborative results, see Tables 6, 7, and 8.

CHLORINE

The Association has an official method for chlorine in plants.[3] Tilden[4] showed the minimum quantity of sodium carbonate required to prevent volatilization of chlorine during incineration.

The chlorine in whites and yolks separated from several samples of eggs was determined in solutions prepared by various procedures; namely, (1) in the ash; (2) after charring at 450° and leaching; (3) after ignition with excess magnesium acetate at 600° for 1 hour; and (4) after ignition with sodium carbonate at 500° for 1 hour. The results found are shown in Table 5.

The results obtained during the 1930 collaborative study of the method for chlorine (NaCl) have been published.[1]

[1] *This Journal*, 14, 412 (1931).
[2] *Ibid.*, 15, 344 (1932).
[3] *Methods of Analysis*, A.O.A.C., 1930, 111.
[4] *This Journal*, 11, 209 (1928).

TABLE 5
Results on chlorine in whites and yolks by various methods.

PROCEDURE OF PREPARING SOLUTION	CHLORINE AS NaCl			
	WHITES		YOLKS	
	per cent		*per cent*	
In ash	0.28	0.25	None	
Charring at 450° and leaching	0.30		0.13	
Ignition at 600° with magnesium acetate	0.18		0.03	
Ignition at 500° with sodium carbonate	0.30	0.30	0.30	0.30
Ignition at 500° with sodium carbonate	0.28	0.28	0.28	0.30

For this year's collaborative results see Tables 6 and 8.

COLLABORATIVE WORK FOR 1932

In order to obviate the uncertainties that may arise through changes taking place in samples of fresh eggs furnished from a central source, the collaborators were asked to obtain their own sample of fresh eggs and to work in pairs. Thus the results of two collaborators in the same laboratory would furnish the best means of judging the reliability of the methods when placed in the hands of different workers. In addition to the methods under study, the collaborators were asked to determine the total solids and the total nitrogen by the official methods as these results were desired in the correlation of the collaborative data.

The sample of spray-dried whole eggs submitted to the collaborators represents a lot of eggs dried under the supervision of L. Jones.

PREPARATION OF THE FRESH EGG SAMPLE

Break 18 eggs which are known to be not over two days old into a quart mason jar, without the loss of egg material and with the removal by means of a policeman of all the whites which may adhere to the inside of the shell. Mix the eggs by means of a malted milk stirrer just sufficiently to break the cellular structure but without producing any foam, then stir with a spatula or spoon. Start the analysis within 2 or 3 hours after breaking out the eggs in order to eliminate possibility of changes taking place in the egg material. Mix the sample by means of a spatula or a spoon before removing portions for analysis.

METHODS OF ANALYSIS

The methods submitted for testing follow:

PHOSPHORUS
PREPARATION OF SOLUTION

Liquid Eggs.—*Methods of Analysis, A.O.A.C.,* 1930, 248.

Dried Eggs.—Transfer 1 gram of the well-mixed sample into a 150 cc. low-form, Pyrex beaker; add 20 cc. of the sodium carbonate solution, and proceed as directed under *Liquid Eggs.*

Fat (Acid Hydrolysis Method)
PREPARATION OF SOLUTION

Liquid Eggs.—*This Journal,* **15,** 313 (1932).

Dried Eggs.—*This Journal,* **16,** 73 (1933).

LIPOIDS AND LIPOID PHOSPHORUS (P_2O_5)

REAGENTS

(a) *Mixed solvent.*—Equal volumes of chloroform and absolute alcohol.

(b) *Alcoholic sodium hydroxide.*—Prepare a saturated sodium hydroxide solution free from carbonates by dissolving 100 grams of sodium hydroxide in 100 cc. of water. Allow to stand until clear, or filter through a hard filter paper which has been soaked in alcohol. (Five cc. of the sodium hydroxide solution contains approximately 4 grams of sodium hydroxide.) Dissolve 50 cc. of the sodium hydroxide solution in 900 cc. of 95 per cent alcohol and dilute with 95 per cent alcohol to 1 liter.

PREPARATION OF SOLUTION

(I) *Liquid Eggs, Washing Modification.*—From the well-mixed sample, weigh accurately by difference approximately 2 grams of yolks, or 5 grams of whole eggs or whites into a 50 cc. beaker, add 5 cc. of the mixed solvent, break up the coagulated material with a stirring rod, and decant through a 7 cm. filter paper into a 250 cc. beaker. Repeat the addition of the mixed solvent in 5 cc. portions, the breaking up of the coagulated material with a stirring rod, and the decantation through the filter paper into a 250 cc. beaker until all the coagulated material is transferred to the filter paper. (The coagulated material should be reduced to a finely divided condition and transferred to the filter paper by the third or fourth washing.) Continue the washings, the breaking up of the coagulated material, and the washing of the edges of the filter paper as directed above until the lipoids are removed (20–25 washings).

Liquid Eggs, Extraction Modification.—Weigh accurately by difference approximately 4 grams of the well-mixed sample into a 100 cc. volumetric flask, and very slowly (drop by drop) from a pipet with constant shaking until the proteins become coagulated and then thoroughly broken up, 25 cc. of the mixed solvent; add 60–65 cc. more of the solvent, and allow to stand 1 hour, mixing at 5 minute intervals. Fill to the mark with the solvent, shake, and pour as rapidly as possible onto an 18½ cm. folded filter, covering the funnel with a watch-glass during filtration, and collecting the filtrate in a 200 cc. Erlenmeyer flask.

Dried Eggs, Washing Modification.—Transfer 1 gram of the well-mixed sample onto a 7 cm. filter, and wash with 5 cc. portions of the mixed solvent, stirring by means of a glass rod the material on the filter paper and washing the edges of the filter paper, until the lipoids are removed (20–25 washings).

Dried Eggs, Extraction Modification.—Transfer 2 grams of the well-mixed sample into a 100 cc. volumetric flask, add 85–90 cc. of the mixed solvent, and allow to stand 1 hour, mixing at 5 minute intervals. Proceed as directed under *Liquid Eggs.*

DETERMINATION

(II) Use all the extract obtained under (I) in the washing modification, or transfer 50 cc. of the filtrate prepared in the extraction modification under (I) to a 150 cc. beaker, and evaporate the extract to dryness on a steam bath (an electric fan, or a gentle blast of dry air, may be used to hasten evaporation). Place the beaker into an oven at 100°C. for 5–10 minutes to remove any remaining moisture. Dissolve the dry extract in 5–10 cc. of chloroform, and filter the solution into a weighed 100 cc. Pyrex beaker through a pledget of cotton packed into the stem of the funnel. Transfer through the funnel into the 100 cc. beaker by means of chloroform from a wash-bottle all soluble extract from Pyrex beaker bottom and sides. Finally wash funnel and stem tip. (The filtrate should be clear.) Evaporate the chloroform on a steam bath, and dry the beaker and contents in an oven at 100°C. to minimum weight (approximately 90 minutes). Allow the beaker to stand in the air until no further change in weight takes place (approximately 30 minutes), and weigh. Report the extract as percentage of lipoids.

LIPOID PHOSPHORUS (P₂O₅)

(III) Dissolve the dried lipoids obtained under (II) in 2–3 cc. of chloroform, add 10–20 cc. of the alcoholic sodium hydroxide solution, evaporate to dryness on a steam bath (care), and place the beaker into an oven at 100°C. for 30 minutes to remove any remaining moisture. Transfer the beaker while hot to an electric muffle heated to 500°C. (faint redness), and allow it to remain at that temperature for 1 hour. Cool, add a few drops of water, break up the charge with a glass rod (flattened end), cover the beaker with a watch-glass, add slowly 5 cc. of nitric acid (1+3), mix, remove the watch-glass, and filter, collecting the filtrate in a 300 or 500 cc. Erlenmeyer flask. Wash thoroughly the charred material and filter paper with water from a wash-bottle.

DETERMINATION

(IV) In the filtrate prepared under (III), determine the phosphorus as directed under 11, 10(b) (*Methods of Analysis*, *A.O.A.C.*, 1930, 16), using 20–50 cc. of the molybdate solution. Report as percentage of lipoid phosphorus (P₂O₅).

DEXTROSE AND SUCROSE

REAGENTS

(a) *Calcium carbonate*.

(b) *Sodium chloride solution*.—Dissolve 50 grams of NaCl in H₂O and dilute to 1 liter.

(c) *Alcohol*.—95 per cent.

(d) *Phosphotungstic acid*.—Powdered.

(e) *Potassium chloride*.—Powdered.

(f) *Sodium hydroxide solution*.—Dilute one volume of sodium hydroxide solution (1+1) with four volumes of H₂O.

PREPARATION OF SOLUTION

(V) *Liquid Eggs*.—Weigh accurately by difference approximately 25 grams of the well-mixed sample into a 250 cc. volumetric flask containing 1 gram of calcium carbonate and 50 cc. of the sodium chloride solution; add with continuous mixing 130 cc. of 95 per cent alcohol. Allow to stand a few minutes for gas bubbles to rise to the surface, cool to room temperature, fill to the mark with water, shake, and filter (18½ cm. folded filter). Transfer 150 cc. of the filtrate to a 250 cc. beaker, evaporate to 20–30 cc. to remove the alcohol, cool, wash with water into a 100 cc. volumetric flask, holding the volume to 80–90 cc.; add in small quantities the dry powdered phosphotungstic acid in slight excess to precipitate any protein, mix, let stand a few minutes for gas bubbles to rise to the surface, fill to the mark with water, shake, and filter. To the filtrate, add in very small portions sufficient dry powdered potassium chloride to precipitate any excess phosphotungstic acid, filter if necessary, and test the filtrate for complete precipitation.

Dried eggs.—From the well-mixed sample, transfer 2.5 grams of whites, or 10 grams of yolks or whole eggs, into a 250 cc. volumetric flask containing 1 gram of calcium carbonate and 50 cc. of the sodium chloride solution, and allow to stand 1 hour, mixing at 5 minute intervals. Add with continuous mixing 130 cc. of 95 per cent alcohol, and proceed as directed under *Liquid Eggs*.

DETERMINATION

(VI) *Reducing sugars direct*.—Transfer 25 cc. of the filtrate prepared under (V) to a 400 cc. beaker, and proceed as directed under XXXIV, 38 (*Methods of Analysis*, 1930, 379). Report as percentage of dextrose.

(VII) *Reducing sugars invert*.—Transfer 50 cc. of the filtrate prepared under

TABLE 6

Collaborative results on laboratory separated liquid whole eggs.

(Two-day-old eggs. Results expressed in percentage.)

Collaborators	Sample Number*	Solids	Nitrogen Total	Water-soluble Total	Water-soluble Crude Albumin	Phosphorus as P_2O_5	Fat (acid hydrolysis)	Lipoids Extraction Total as P_2O_5	Lipoids Extraction Phosphorus	Lipoids Washing Total as P_2O_5	Lipoids Washing Phosphorus	Reducing Dextrose	Sucrose Added 1.0%	Sucrose Recovered 10.0%	NaCl	Salt Added 1.0%	Salt Recovered 10.0%
Haenni	1	25.97	2.04	1.20	0.90	0.50	11.66	13.12	0.34	12.85	0.34	0.33	1.02	10.21	0.29	0.99	9.99
		25.97	2.04	1.19	0.90		11.92	13.10	0.35	12.78		0.34			0.29	1.00	10.10
			2.05														
Pruitt	1	25.95	2.05	1.21	0.91	0.50	11.77	12.98	0.36	12.79	0.34	0.27			0.29	0.96	9.93
		25.97	2.05	1.21	0.91	0.49	11.64	13.08	0.36	12.69	0.34	0.26			0.28	1.00	10.01
			2.06														
Buell	2	25.82	2.07	1.24	0.91	0.47	11.02	12.72	0.34			0.35	1.03	10.02	0.28	1.00	10.13
		25.86	2.08	1.25	0.94	0.48	11.05	12.78	0.34				1.03	10.02	0.29		10.29
Jones	2	25.81	2.08	1.25	0.95	0.48	11.15	12.74	0.33			0.33	1.01	9.99	0.31	0.99	10.00
		25.78	2.08	1.25	0.94	0.48	11.18	12.85	0.34			0.32	0.98	9.97	0.32	0.97	
Stark	3	25.93	2.08	1.19	0.92	0.51	11.28	12.94	0.36			0.31	1.00	10.10	0.27	1.01	9.95
		25.86	2.08	1.18	0.90	0.50	11.21	12.90	0.36			0.31	0.97	9.98	0.27	1.00	9.91
		25.84	2.07	1.19	0.90	0.50	11.18	12.82	0.35								
Stone	3	25.81	2.06	1.23	0.92	0.50	11.03	12.88	0.36			0.37			0.27	0.96	9.95
		25.82	2.06	1.23	0.94	0.50	11.03	12.92	0.37			0.37			0.29	0.98	9.99
		25.85	2.07	1.24	0.94	0.50	11.03	12.94	0.38								

* Sample 1.—Eggs laid March 21, 1932.
Sample 2.—Eggs laid during May 1932.
Sample 3.—Eggs laid June 5, 1932.

(V) to a 100 cc. volumetric flask, add 5 cc. of strong hydrochloric acid, and allow to stand overnight. Neutralize with the sodium hydroxide solution, cool to room temperature, and fill to the mark with water. Transfer 50 cc. (or less) to a 400 cc. beaker, and proceed as directed under XXXIV, 38 (*Methods of Analysis, A.O.A.C.*, 1930, 379). Deduct the percentage of invert sugar obtained before inversion from that obtained after inversion, multiply the difference by 0.95, and report as percentage of sucrose.

WATER-SOLUBLE NITROGEN AND WATER-SOLUBLE NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL (CRUDE ALBUMIN NITROGEN)

The term "crude albumin nitrogen" is used to designate the water-soluble nitrogen precipitable by 40 per cent alcohol.

This Journal, 15, 75 (1932).

CHLORINE

Methods of Analysis, A.O.A.C. 1930, 249

TABLE 7

Collaborative results on commercially separated liquid whites, yolks, and whole eggs; (March, 1932, Current Receipt Eggs.)

| COLLABORATORS | SAMPLE NUMBER* | EGG SUBSTANCE | SOLIDS | NITROGEN | | PHOSPHORUS AS P_2O_5 | FAT (ACID HYDROLYSIS) |
				TOTAL	WATER-SOLUBLE		
			per cent	*per cent*	*per cent*	*per cent*	*per cent*
McNall	1	Whites	12.21	1.74	1.63		
Mitchell		Whites	12.24	1.76	1.64		
McNall	2	Whites	12.36	1.75	1.66		
Mitchell		Whites	12.35	1.75	1.65		
McNall	1	Yolks	46.75	2.54	0.64	1.22	28.42
Mitchell		Yolks	46.73	2.52	0.63	1.22	28.62
McNall	2	Yolks	45.57	2.52	0.62	1.20	27.75
Mitchell		Yolks	45.61	2.46	0.62	1.19	27.76
McNall	1	Whole	27.52	2.16	1.23	0.55	12.40
Mitchell		Eggs	27.47	2.17	1.25	0.55	12.43
McNall	2	Whole	26.65	2.13	1.18	0.55	12.07
Mitchell		Eggs	26.62	2.08	1.19	0.53	12.02

* Sample 1—Whites and yolks separated from the same eggs. Whole eggs represent the same lot of eggs. Samples 2 were collected in the same manner as was Sample 1.

Collaborator McNall, Chicago, made his study of the methods for reducing sugars, with and without added sucrose, and for chlorine, with and without added salt, on commercially separated yolks. He reported 0.19 per cent dextrose, and 1.07 and 9.85 per cent sucrose (added 0.96 and 9.16 per cent, respectively); and 0.29 per cent chlorine as NaCl and 1.23 and 9.35 per cent salt (added 1.24 and 9.38 per cent, respectively).

TABLE 8

*Collaborative results on commercially spray-dried whole eggs.**

COLLABORATORS	SOLIDS	WATER-SOLUBLE NITROGEN		PHOSPHORUS AS P₂O₅	FAT (ACID HYDROLYSIS)	LIPOIDS MODIFICATION OF METHOD				DEXTROSE	CHLORINE AS NaCl
						EXTRACTION		WASHING			
		TOTAL	CRUDE ALBUMIN			TOTAL	PHOSPHORUS AS P₂O₅	TOTAL	PHOSPHORUS AS P₂O₅		
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Haenni	96.35	4.09	3.10	1.94	44.44	48.35	1.31	47.67	1.29	0.57	1.05
	96.33	4.09	3.08	1.94	44.22	48.44	1.31	47.59	1.29	0.54	1.06
		4.08	3.07								
Jones		4.78	3.75	1.97	43.32	48.53	1.34			0.56	1.05
		4.40	3.44	1.96	43.37	48.46	1.34			0.56	1.03
				1.95	43.75					0.61	1.00
McNall		4.30	3.23	2.00	43.75	47.52	1.31	47.31	1.32	0.45	1.02
		4.30	3.23	2.00	43.72	47.54	1.31	47.33	1.33	0.44	1.02
Stone		4.91	3.68	2.01	43.44			48.21	1.32	0.56	1.05
		4.94	3.71	2.01	43.47			48.23	1.32	0.51	1.05
		4.95	3.74	2.03	43.51			48.25	1.33	0.59	1.08
					43.55			48.27	1.33		1.08
Jones†	96.71	4.67	3.66	1.97	44.03					1.21	1.03

* This sample was prepared under Jones's supervision during July 1931. His results on the liquid eggs used in the preparation of the dried eggs, calculated in the dry substance, are: Total nitrogen 7.79 per cent; water-soluble nitrogen 4.35 per cent; crude albumin nitrogen 3.36 per cent; phosphorus (P₂O₅) 1.96 per cent; fat 45.87 per cent; dextrose 1.18 per cent; and chlorine (as NaCl) 1.07 per cent.

† Jones's results were obtained at the time the dried eggs were prepared. He obtained 7.78 per cent total nitrogen (calculated to the dry substance).

COMMENTS BY COLLABORATORS

Haenni.—The extraction modification for the determination of lipoids gives somewhat higher results than the washing modification.

Pruitt.—Sugar recovery: After addition of phosphotungstic acid, the analysis had to wait until next day. A considerable amount of sugar was inverted. The results are therefore not given.

Stark and Stone.—C. W. Harrison, Chief, Minneapolis Station, in transmitting the collaborative results states: We note that in the case of the addition of cane sugar some inversion apparently took place so that the amount of cane sugar recovered was less than the amount added. The total reducing sugars, however, figured as sucrose, after correcting for the amount of reducing substances occurring in the eggs totals approximately the percentage of sucrose added, showing that a partial inversion of the cane sugar did take place. This sugar was added, and the samples held in the ice box overnight before that analysis was completed.

Stone.—(Dried Eggs). Under the method for fat by acid hydrolysis I believe a counterpoise flask should be used in weighing up the fat. Changes in the laboratory humidity would affect the weight considerably.

Under Lipoids and Lipoid P_2O_5 I used the washing modification on a 1 gram sample. The same comment could be applied here on weighing a beaker without a counterpoise. Atmospheric conditions would give different weights depending upon the humidity.

Under Preparation of Solution for the water-soluble nitrogen determinations some modification might be used. Mixing the sample with water after the fat extraction seems rather a difficult procedure and still avoid the emulsion difficulties. Would it be advisable to break up the sample with a long stirring rod as the water is added gradually to the sample. It seems rather difficult to secure a clear solution after the filtering process. Under the term "centrifugalize" I believe a time limit should be given or the analyst should spin the bottles until a clear solution is obtained.

McNall.—In the method for sucrose on liquid eggs my results would indicate that some correction should be made for the volume of insoluble egg material when 25 grams is made up to 250 cc. The amount of sucrose found was in excess of the calculated amount in both cases.

In the method for water-soluble nitrogen I had trouble getting a clear filtrate, and the filtration was very slow. To make the method comparable to liquid eggs it would be necessary to add 10–15 cc. of 0.01 N acetic acid per gram of egg material.

DISCUSSION

Total phosphorus (P_2O_5), fat, lipoids, lipoid phosphorus (P_2O_5), reducing sugars (dextrose), water-soluble nitrogen, crude albumin nitrogen, and chlorine were determined on three samples of fresh whole eggs by six collaborators, two collaborators making the determinations on each sample of eggs. In addition, each collaborator added known quantities of sugar and salt to portions of the samples and determined the added materials. Further, each collaborator determined the solids and the nitrogen in accordance with the official methods. The results are given in Table 6.

The agreement in results for all the determinations between each pair of collaborators is remarkably close. With exception of the results for fat and dextrose, the agreement in results becomes even more remarkable when comparison is made of the collaborative results for all three samples

of eggs. The agreement in results affords further evidence of the uniform composition of eggs (*This Journal* 15, 310 (1932).

The variation in results for reducing substances (dextrose) is probably explained by the fact that the reducing substances may disappear from the eggs within a comparatively short time after the eggs are broken out; hence, the relatively short interval between the time the two collaborators had this determination under way may be sufficient time for a slight decomposition to take place, and to cause the variation in the results reported.

While most of the collaborators obtained for fat closely or fairly closely agreeing results individually or on the same sample of eggs, the collaborative results when compared for all three samples of eggs are not so uniform as are the results for the other determinations. With the results for solids, nitrogen, and phosphorus (P_2O_5) on all three samples of eggs running well within the analytical error of the individual analyst, there appears to be no reason to expect the somewhat wider variation for fat. The results for lipoids, which consist essentially of the same substances as the fat by acid hydrolysis, except the lecithin is not broken down in the determination, show less than half the variation as reported for fat. Further, the fat obtained by acid hydrolysis appears to differ in composition since its acidity varies from sample to sample. The free fatty acids were determined as directed by the official method[1] in the fatty residues of 55 samples of yolks. The results varied from 9.8 to 15.3, averaging 12.05 cc. of 0.1 N NaOH per gram of fat. It may be that the writer is expecting more of the method than it is capable of yielding in the hands of different analysts, or for that matter in the hands of the individual analyst for check determinations on the same sample at different times. It appears that further study of the method is desirable.

Table 7 gives the results of two collaborators working on commercially prepared whites, yolks, and whole eggs. Water-soluble nitrogen, phosphorus (P_2O_5), and fat were the only determinations made in the collaborative study of the methods. All the results are in close agreement.

Table 8 shows the collaborative results reported on a sample of commercial spray-dried whole eggs. The results for phosphorus (P_2O_5), fat, lipoids, lipoid phosphorus (P_2O_5), reducing sugars (dextrose), and chlorine appear to be satisfactory. On water-soluble and crude albumin nitrogen, two of the collaborators obtained results which check closely the results obtained on the original liquid eggs when the results are calculated to the same solid basis. The other two collaborators obtained results for these determinations which are appreciably higher than the results obtained on the liquid eggs, when calculated to the same solid basis. As the method is not yielding satisfactory results in the hands of different analysts, further work should be done on it. McNall suggests that the added acetic

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[1] *Methods of Analysis*, A.O.A.C., 1930, 326.

acid be increased to correspond more closely with the amount added to liquid eggs on the basis of the solid content.

Using glass beakers instead of platinum dishes in the determination of phosphorus and chlorine, it is no longer necessary to add olive oil to prevent frothing.

The extraction modification on the method for lipoids requires much less actual working time of the analyst and seems to be preferred by the collaborators instead of the washing modification of the method. There appears little difference in the results. The chief objection to the extraction modification in the past has been the probable loss of the solvent during filtration. It appears that this objection can be overcome by eliminating the filtration. The insoluble egg material settles, leaving a clear solution from which an aliquot free from insoluble material can be withdrawn. In most extraction methods where volumetric flasks are used, it no longer appears customary to correct results due to the volume occupied by relatively small amounts of insoluble residues.

In the dextrose determination, however, particularly on liquid yolks, the volume occupied by the insoluble residue causes the results to be approximately 7.5 per cent too high. On dried yolks and whole eggs, the results are appreciably high. On commercially prepared dried eggs, the dextrose determination is of little or no value, except for detection of adulterants, since the dextrose is fermented out before the drying takes place. Since the amount of reducing substances occurring naturally in yolks is small, there appears to be no urgent need to make a relatively small correction due to the volume of insoluble residue. When relatively large amounts of sugar are added, as is frequently the case in commercially prepared frozen yolks, it seems desirable that the method should carry a correction factor. This matter is left to the decision of the Association.

RECOMMENDATIONS[1]

It is recommended—

(1) That the tentative method for the determination of total phosphorus (P_2O_5) (*Method of Analysis, A.O.A.C.*, 1930, 248), as revised by the associate referee, be adopted as official (first action); that the heading of the method be changed from "Phosphoric-Pentoxide" to "Total Phosphoric acid"; and that "(b) *Olive Oil*" under Reagents and the sentence "Add to the whites 0.5 cc. of olive oil" under Preparation of Solution be deleted.

(2) That the method for the determination of fat by acid hydrolysis (*This Journal*, 15, 313 (1932), as revised by the associate referee, be adopted as tentative and further studied collaboratively.

(3) That the extraction modification of the method for the determination of lipoids and lipoid phosphoric acid described in this report be

adopted as tentative and further studied collaboratively after deleting "and pour as rapidly as possible onto an 18½ cm. folded filter, covering the funnel with a watch-glass during the filtration, and collecting the filtrate in a 200 cc. Erlenmeyer flask" and substituting "allow to stand until clear, and transfer a 50 cc. aliquot to a 250 cc. beaker."

(4) That the method for the determination of reducing sugars and sucrose, as given in the associate referee's report, be adopted as official (first action).

(5) That the tentative method for the determination of water-soluble nitrogen and water-soluble nitrogen precipitable by 40 per cent alcohol (crude albumin nitrogen) (*This Journal*, 15, 75 (1932) on liquid eggs be adopted as official and on dried eggs be further studied.

(6) That the tentative method for the determination of chlorine (*Methods of Analysis*, A.O.A.C., 1930, 249) be adopted as official (first action); and that "**(b)** Olive Oil" under Reagents be deleted.

No report on detection of decomposition was given by the associate referee.

The report on glycerol and unsaponifiable matter was given. See report of the Referee on Eggs and Egg Products, who also acted as Associate Referee on Glycerol, etc.

REPORT ON PRESERVATIVES

By JOHN C. KRANTZ, Jr. (State of Maryland Department of Health, Baltimore, Md.) *Referee*

(1) Attention was called to the determination of boric acid, *Methods of Analysis*, 1930, p. 340, by Leslie Hart. The method directs the use of 10 grams of mannitol for each titration. This quantity is excessive; mannitol is an expensive reagent and, besides, in the method for the titration of boric acid in fertilizers only 1 to 2 grams is required.

The referee recommends therefore that the quantity of mannitol directed to be used in the titration of boric acid in food preservatives be changed from 10 grams to 2 grams.

(2) A new quantitative procedure for the determination of saccharine by Gales and Pensa of the New York City Department of Health was studied. The present official method depends upon the conversion of the sulfur present in the molecule to sulfate and its estimation as barium sulfate. Commercial saccharine may be contaminated with para-sulfonic benzoic acid. This is estimated as saccharine by the official method.

Gales and Pensa recommend a modification of the method suggested

by Giuseppi.[1] The method depends upon the hydrolysis of saccharine into ammonia by evaporation in the presence of hydrochloric acid. Para-sulfonic benzoic acid does not form an imide and hence will not respond to the test. The resulting ammonium chloride is determined by comparison with standard ammonium chloride solutions after nesslerizing.

The method is expeditious and lends itself readily to the determination of saccharine in beverages. In this laboratory satisfactory results were obtained; however certain minor modifications of the procedure might be found necessary.

The referee recommends that the Gales-Pensa method be submitted for collaborative work.

(3) Reports on Special Preservatives for Foods:

(a) British Pat. 339,602.—E. Schultze suggests the use of quinatoxines with benzoic acid for the purpose of preservation and sterilization of various products.

(b) French Pat. 695,096.—The investigator observed that foods are preserved by heating them in a solution of salts of open chain hydroxyacids, which facilitates the swelling of dried products. Examples are given of the use of sodium lactate and gluconate.

(c) Ethylene oxide has been used as a food preservative. Sudendorf and Kröger[2] point out the dangers involved in the use of this substance. Its toxicity should be thoroughly investigated.

(4) The esters of *p*-hydroxy benzoic acid:

The esters of *p*-hydroxy benzoic acid are being recommended generally as preservatives for food and pharmaceutical preparations. During the last half decade much interesting data have been published regarding the efficacy of these compounds as disinfectants and preservatives. Various esters have been marketed under trade names. Perhaps the best known of these are "Nipagin" and "Nipasol."

Sabalitschka[3] made a pharmacological investigation of the methyl ester of *p*-hydroxy benzoic acid and found it harmless and non-irritating. The phenol coefficient was determined to be 17. This worker recommends the use of this ester in acid, neutral or alkaline medium, in concentrations from 0.025 to 0.15 per cent as a preservative. Later this investigator[4] determined the toxicity of the ester to human beings and found it to be 75 per cent less noxious than benzoic acid. A further investigation[5] confirms the foregoing report. The ratio of the toxicity to the antiseptic dose was determined. The dog was used as a test object. For benzoic acid the ratio is 3.3; for the methyl ester of *p*-hydroxy benzoic acid the ratio is 50.

Some years ago Weisz[6] developed a method for the determination of this ester in foods. The increasing number of references to the compound in the literature warrants study of it as a food preservative.

[1] *Z. Nahr. Genussm.*, **18**, 577 (1909).
[2] *Chem. Ztg.*, **55**, 549 (1931).
[3] Pharm. Weekblad, **68**, 947 (1931).
[4] *Volksernährung*, **5**, 247 (1930).
[5] Anon., *J. Pharm. Chem.*, **123**, 638 (1931).
[6] *Z. Untersuch. Lebensm.*, **55**, 24 (1928).

The referee recommends that a survey be made to determine whether this compound is being used extensively in this country as a food preservative. If the use warrants it, it is further recommended that the Weisz tests be submitted for collaborative study.[1]

The report of J. Fitelson, Associate Referee on Sulfurous Acid in Dried Fruits, will be presented as a separate communication. This report also embraces certain recommendations for changes in the text of *Methods of Analysis* regarding the Monier-Williams determination of sulfurous acid.

REPORT ON SULFUROUS ACID IN DRIED FRUITS

By J. FITELSON (U. S. Food and Drug Administration, New York, N. Y.), *Associate Referee*

There is no method for the determination of sulfurous acid in dried fruits which can be said to be accurate. Since the sulfurous acid content of dried fruits is unknown, the method that combines high yields together with rapidity and ease of manipulation may be considered as the best available. Studies by Fitelson,[2] Nichols and Reed,[3] and May[4] indicate that the Monier-Williams method[5] gives a greater recovery of sulfur dioxide than do other methods. Monier-Williams[6] and Fitelson[2] have also shown that longer distillation (1½ hours) is necessary with dried fruits than with other products. In view of these considerations, and the fact that a thorough search of the literature suggested no other mode of attack, no work was done on the subject.

H. A. Lepper has called attention to the misleading arrangement of methods for sulfurous acid in *Methods of Analysis*. For the sake of clarity, the Monier-Williams method should be placed under the heading "Total Sulfurous Acid" instead of under "Free Sulfurous Acid," also the statement "Applicable in the presence of other volatile sulfur compounds" should be incorporated in the Monier-Williams method since the distillation method (par. 31, p. 343) is "not applicable if volatile organic sulfur compounds may be set free." Volatile sulfur compounds, inorganic as well as organic, do not interfere appreciably with the Monier-Williams method.

It is therefore recommended[7]—

(1) That the Monier-Williams method be adopted as a tentative method for sulfurous acid in dried fruits, with the sentence " * * * boil the mixture for 1 hour," * * * line 1, page 345, *Methods of Analysis*, 1930, changed to read " * * * boil the mixture for 1½ hours * * *."

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 57, 77 (1933).
[2] *This Journal*, 12, 120 (1929).
[3] *J. Ind. Eng. Chem. Anal., Ed.*, 4, 79 (1932).
[4] *Analyst*, 52, 526 (1927).
[5] *Methods of Analysis, A.O.A.C.*, 1930, 344.
[6] Reports on Public Health and Medical Subjects, No. 43. London (1927).
[7] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 57 (1933).

(2) That the subject of sulfurous acid in dried fruits be dropped until some method yielding higher results than the Monier-Williams method is presented.

(3) That par. **34**, p. 344, *Methods of Analysis*, 1930, entitled "Monier-Williams Method—Tentative" be placed under the general heading "Total Sulfurous Acid," page 343.

(4) That the following statement be placed under the title "Monier-Williams Method—Tentative," par. 34, p. 344: "(Applicable in the presence of other volatile sulfur compounds)."

REPORT ON COLORING MATTERS IN FOODS

By C. F. JABLONSKI (U. S. Food and Drug Inspection Station, New York, N. Y.), *Referee*

In compliance with the Association's request, the referee sent six sets of seven samples each of noodles to six collaborators for the identification of coloring matter.

The purpose of this work was to test out a method submitted by the referee for the separation and identification of both vegetable and coal tar coloring matter which might occur in this sort of product. The detection of vegetable colors in alimentary products is of special importance, since they may easily be overlooked owing to the fact that vegetable coloring matters do not as a rule give the characteristic reactions of the coal tar dyes.

The method is based primarily upon separating the various coloring matters with different immiscible solvents into definite groups. Methods of identification and reactions are described for each group.

The samples of noodles were colored as follows: (1) annatto, (2) tartrazine and orange I, (3) turmeric, (4) naphthol yellow S and orange I, (5) saffron, (6) yellow OB, and, (7) egg yolk.

Only three reports were received from the collaborators. The results follow:

K. Breen	(1)—Annatto
Dept. of Agr. & Markets	(2)—No report
Albany, N. Y.	(3)—Turmeric
	(4)—Naphthol Yellow S and Orange I
	(5)—Saffron
	(6)—Yellow O B
	(7)—Natural Plant Pigment
W. C. Woodfin	(1)—Annatto
Food & Drug Adm.	(2)—Tentatively identified as naphthol yellow S, but
Baltimore, Md.	not satisfied with determination.
	(3)—Turmeric
	(4)—Naphthol yellow S

(5)—Saffron
(6)—Yellow O B
(7)—Not completed

C. H. Hickey   (4)—Naphthol yellow S and orange I.
Food & Drug Adm.
Boston, Mass.

COMMENTS

The following comments and criticism were offered by the collaborators.

K. Breen.—It is noted that the petroleum ether is not colored in the first extraction when turmeric is present and consequently the fourth sentence of the first paragraph is misleading, because turmeric remains in the original aqueous extract. Hence when washed with dilute ammonia water (1+50), acidified, and extracted with ethyl ether, it fails to show the characteristic turmeric tests. Also, in the next to last sentence in paragraph I, the question arose as to whether the alkaline aqueous solution freed from fat and oil-soluble coal tar dyes should be added to the original aqueous solution mentioned in the first sentence of the second paragraph.

W. C. Woodfin.—The method submitted did not give very satisfactory results. This might be due in part to my limited experience with dyes. After extraction with 80 per cent alcohol and evaporation, there was always a pasty mass present which interfered with the separation.

1. The test given for the initial identification of yellow AB and yellow OB did not give very satisfactory results, possibly due to too great a dilution of the dye.

2. There was no difficulty experienced with the identification of annatto and saffron. However, in the case of turmeric it was found necessary, even when working with known turmeric, to increase the concentration of the HCl quite materially in order to get the red color. (I got the red color quite well using equal volume of the ether extract and concentrated HCl.) It will be noted that the boric acid test for turmeric as given is not complete, but I understood that you add a few crystals of boric acid.

3. As I did not find either of the oranges or martius yellow to be present in any of the samples, I cannot give any appraisal of the method given for these. The naphthol yellow S can be identified largely by its disappearance and reappearance in acid and alkaline solution respectively.

DISCUSSION

If consideration is given to this fragmentary report, it will appear that the collaborators were quite successful in separating the various coloring matters from their respective immiscible solvents and encountered no special difficulties in identifying them. In only one instance was an erroneous identification made, but the collaborator himself was not satisfied with the determination. In another instance an omission was made. The balance of the report was correct.

Some allowance should also be made for the limited experience of the collaborator in working with coloring matters.

The referee would therefore suggest additional collaborative work and more cooperation on the part of the collaborators.

RECOMMENDATIONS[1]

It is recommended—

(1) That additional collaborative work be devoted to the separation and identification of coloring matters in alimentary paste.

(2) That the study of the quantitative separation and estimation of the recently permitted dyes be continued.

(3) That additional investigation be carried out on the qualitative separation of light green SF yellowish, brilliant blue FCF, and fast green FCF.

REPORT ON METALS IN FOODS

By H. J. WICHMANN (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

Associate referees were appointed this year for tin, lead, copper and zinc, and arsenic in foods. The referee will discuss these subjects according to their relative importance and interest, the subject of greatest interest being considered last.

TIN

No report was made by the Associate Referee on Tin. The interest of members of the Association in this subject has been gradually ebbing, therefore the referee can see no advantage to the Association in supplying an artificial stimulus, although he acknowledges that the methods could be improved. If any member is interested in this subject the referee would be glad to cooperate in further work.

LEAD

The associate referee continued the work begun last year on the colorimetric determination of lead by means of the diphenyl carbazide reaction with chromium after a precipitation of lead chromate. He reports results from two collaborators, and on the basis of these reports recommends that the method be adopted as a tentative method. The referee does not agree with this recommendation because the results are too limited in number, and the average is only about 90 per cent of theory. Of the three variations of the method tried, the one with the most precipitations gave the lowest results. No chemical substance is absolutely insoluble, therefore if the conditions selected for lead determinations are not the optimum, losses will occur, as seemed to be the case here. One of the sources of loss may be during the sulfide precipitation, which is made in acid solution, but no mention is made of an optimum pH. If the precipitation is made at too low a pH all the lead may not be precipitated; if the pH of precipitation is too high, elements that may later interfere with the

zeit. The quantities of arsenic used were 0.003, 0.010, 0.012, 0.020 and 0.030 grains. This enabled all chemists who had not used either or both methods to become familiar with the technic.

Second Stage.—An attempt was made to simulate the conditions under which a sample of apples would be run and also to have definite quantities of arsenic present. Dried apple peelings from apples which had never been sprayed and a standard arsenic solution were sent to the collaborators. They were instructed to digest 15 grams of peelings, equal to peelings from one pound of apples, and to take definite amounts of the digested solution, equal to 0.003, 0.010 and 0.030 grains of As_2O_3 per pound of fruit, respectively. The two methods were to be run as directed in the first stage.

TABLE 1—*First Stage*

Results of determinations made on apple pulp with known quantities of arsenic solution.

			GUTZEIT METHOD†		
THEORY	grain 0.003	grain 0.010	grain 0.012	grain 0.020	grain 0.030
Collaborator*					
1		.0094	.0119	.0235	(.0297)
3	.0036	.0093	.0097	.0184	(.0281)
6	.0040	.0103	.0117	.0210	.0301
8	.0035	.0078	.0100	(.0165)	(.0252)
10	.0032	.0112	.0151	(.0217)	.0269
11	.0037	.0103	(.0110)	.0213	.0247
12	.0033	(.0106)	(.0123)	.0136‡	(.0201)‡
Average	.0035	.0100	.0117	.0204	.0275
			BROMATE METHOD†		
THEORY	0.003	0.010	0.012	0.020	0.030
Collaborator*					
1		.0098	.0116	.0119	.0298
3	.0024	.0101	.0117	.0199	.0293
6	.0044	.0104	.0121	.0188	.0295
8	.0031	.0106	.0126	.0199	.0298
10	.0047	.0121	.0142	.0207	.0296
11	.0027	.0083	.0100	.0203	(.0267)
12	.0042	(.0115)	.0118	.0199	.0293
Average	.0036	.0104	.0120	.0199	.0291

* Only eight collaborators reported the preliminary work, one of whom, No. 9, used aliquots different from those recommended. His results were good by both methods. These collaborators used their own standard solutions.

† All Gutzeit figures are averages of six results, and all bromate figures are averages of three results.   ‡ Omitted from average.

( ) Results of which this figure is an average were not uniform. Varied from average 0.003 grain.

— Varied from theory at least 10%.

⸻ Varied from theory at least 20%.

TABLE 2—*Second Stage*

Results of determinations made on apple peelings with definite quantities of standard arsenic solution.

THEORY	GUTZEIT METHOD			BROMATE METHOD		
	grain 0.003	grain 0.010	grain 0.030	grain 0.003	grain 0.010	grain 0.030
Collaborator						
1	.0029	.0093	.0283	.0029	.0099	.0298
2	.0020	.0110	.0330	.0031	.0103	.0310
3	.0020	.0069	.0269	.0029	.0098	.0301
4	.0030	.0080	(.0175)‡	.0030	.0102	.0312
5	.0023	.0093	.0240	.0027	.0097	.0277
6	.0027	.0093	.0284	.0031	.0098	.0301
7	.0034	.0136	.0269	.0024	.0090	.0241‡
8	.0027	.0107	.0360‡	.0030	.0096	.0293
9	.0026	.0095	.0284	.0031	.0113	.0282
10	.0034	.0108	(.0306)	.0037	.0102	.0299
11	.0030	.0080	.0303	.0028	.0094	.0297
12	.0049‡	.0077	(.0192)‡	.0030	.0068‡	.0321
13	.0020	.0060	.0293	.0030	.0100	.0293
14	.0033	.0107	.0305	.0031	.0101	.0300
Av.	.00273	.00936	.02906	.00298	.00989	.02992

 ᵃ See Table I for reference notes.

The results of the various collaborators, and notes pointing out the consistencies, inconsistencies, and variations from the average of both methods are given in the tables and discussion.

DISCUSSION

Possible Interference of Other Spray Materials.—Experiments were tried on fruit sprayed with the following materials to ascertain if there is any interference when either method is used: Bordeaux, borax, lime, nicotine, sulfur, calcium sulfide, barium fluosilicate, and sodium fluoaluminate or cryolite. There was no indication of any interference by any of these materials except in the case of lime sulfur which gave a little higher blank in the bromate method which was lowered to proper limits by decreasing the amount of material used. It is presumed that the amount used commercially will have no effect. A sample sprayed with calcium sulfide one month before testing showed no interference. The samples containing nicotine showed slightly lower results, but not low enough to be conclusive of any interference. However, only 0.01 gram of nicotine sulfate was used as it was not thought likely that a quantity larger than this would be encountered on spray residues on fruits. No work was done with large quantities of nicotine present.

Immersion Method for Dissolving Arsenic.—Work to test the accuracy of the so-called rapid or immersion method was limited to apples which had been sprayed with lead arsenate and later with barium fluosilicate and sodium fluoaluminate. Not all the arsenic was removed, as was proved by digesting the apples after immersion. This may have been due to some extent to occlusion. Further work on this method will be done before the next meeting if possible.

Analytical Method Preferred by Collaborators.—Several collaborators stated a liking for the bromate method except that they prefer to avoid acid digestion of the sample. Others thought the bromate method more accurate but considered the Gutzeit method more rapid. Three stated a definite preference for the bromate and several noted greater consistency. Several pointed out that if it was necessary to repeat an analysis by the bromate method, a new digestion must be made. (In this connection it might be well to mention that a check on the bromate method can be made by determining the arsenic in the distillate by the Gutzeit method.)

Zinc and Paper Used in Gutzeit Method.—Practically all the collaborators used Hanford-Pratt papers. About one-third used stick zinc and most of the others used 30-mesh zinc.

Experiments with Hydrogen Evolution.—Experiments were made to determine the rate of hydrogen evolution with various kinds of zinc over a period of 90 minutes. When a hydrochloric acid concentration was used, as called for in the A.O.A.C. Gutzeit method, 30-mesh zinc evolved hydrogen much too rapidly at the beginning of the reaction and slowed up toward the end. Both the electrolytic stick zinc and the special arsenic-free stick zinc had a practically constant evolution rate during the entire period. They also evolved much less hydrogen than the 30-mesh as shown by the following figures:

	cc.
2 grams of 30-mesh evolved	475
1 gram of 30-mesh evolved	300
5 pieces of electrolytic sticks evolved	165
3 pieces of special sticks evolved	190

When the electrolytic or special arsenic-free stick zinc was used Gutzeit stains were complete in about an hour and they had sharp, well-defined end points.

Modifications of the Bromate Method.—A solution of urea was used with the ammonium oxalate in expelling oxides of nitrogen after digestion. The solution used was a saturated solution of ammonium oxalate containing 50 grams per liter of urea. Fifty cc. for each digestion was used. All products of the reaction are driven off, along with all oxides of nitrogen. It

was deemed best to boil the H_2SO_4 until fumes were up in the neck of the flask. Two thousand samples were run in actual laboratory practice without any apparent inconsistencies.

When the volume of water in the receiving flask was reduced from 150 to 100 cc., all $AsCl_3$ distilled over when the temperature of solution reached 90°C. This smaller quantity of water minimizes the interference of sulfur compounds derived from the H_2SO_4, resulting in a lower and more consistent blank.

Experience has shown that in a laboratory equipped for making 30 digestions and 10 distillations simultaneously, 120 determinations can be made by the bromate method in an 8 hour day by three chemists and one helper. Digestion is complete in 1 hour when the proper equipment is used. The record time for completion of one determination is $1\frac{1}{4}$ hours.

CONCLUSIONS

Both the results of the collaborators and the work of the associate referee show—

(1) That the bromate method is more consistent than the Gutzeit method. This is indicated by the fact that in Table 1 nine Gutzeit results varied more than 0.003 grain from the average, and only two such variations occurred in the bromate results. In Table 2 there are four such results by the Gutzeit method and none by the bromate.

(2) That the bromate method is more accurate than the Gutzeit. This is indicated by the following tabulation:

| | TABLE 1 | | TABLE 2 | |
	10% Error	20% Error	10% Error	20% Error
Gutzeit	17	7	25	15
Bromate	10	5	6	3

The final averages of both methods agree very closely with the theory, but the bromate averages are a little closer.

(3) That the bromate method is more accurate on quantities as small as 0.003 grain. This conclusion is rather surprising as it was expected that the Gutzeit method would be more accurate in the low ranges.

It is recommended[1] that the bromate method, with the modifications mentioned in this report, be adopted as a tentative method for the determination of arsenic residue on fresh fruits (apples, pears, or similar products). This method has been published.[2]

No report on tin was given by the associate referee.

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 58, (1933).
[2] *This Journal*, 16, 75 (1933).

REPORT ON COPPER AND ZINC

By R. M. MEHURIN (Bureau of Animal Industry, Washington, D. C.),
Associate Referee

The referees on metals in foods have since 1913 recommended at various times the collaborative study of the tentative method for the determination of zinc in foods, but a search of the literature discloses no record of any such work having been performed. Therefore, the newly appointed referee on copper and zinc in foods thought it advisable to undertake this work without further delay.

As experience has shown that the errors attendant on the use of a Gooch crucible in the filtering, washing, and ignition of the zinc sulfide are quite large for the smaller quantities of zinc, for example 2 mg. and under, another method was devised. This method follows the regular tentative procedure up to the point of final precipitation of the zinc sulfide. The solution containing the sulfide was then shaken vigorously with quantitative paper pulp, equivalent in amount to one-half of a 7 cm. paper, and filtered through a quantitative paper. The sulfide was then dissolved in a definite quantity of hydrochloric acid (5 cc. 3 N), boiled to remove hydrogen sulfide, and the zinc solution was made up to a definite volume. A one-tenth aliquot was then exactly neutralized with potassium hydroxide, litmus paper being used and care taken to avoid adding an excess of the reagent. One cc. of 0.1 N hydrochloric acid and 1 cc. of 2 per cent potassium ferrocyanide were then added, the solution made up to 50 cc., and the resultant turbidity compared to standard tubes containing the same quantities of potassium chloride (0.10 gram), hydrochloric acid (1 cc. of 0.1 N), potassium ferrocyanide (1 cc. of 2 per cent), and quantities of zinc from 0.05 to 3.0 mg. in increments of 0.05 mg.

Two solutions were sent to nine collaborators with the request that copper be determined by the recently adopted tentative method, the zinc in solution "A" by the above described nephelometric method, and the zinc in solution "B" by the tentative method. These solutions contained the average amount (1%) of ash constituents found in the most commonly used foods and, in addition, solution "A" contained 0.018 mg. of zinc and 0.0036 mg. of copper per cc. and solution "B" contained 0.090 mg. of zinc and 0.0360 mg. of copper per cc.; 50 cc. of solution was taken in each case. Eight replies were received with the following results:

ANALYST	Zn FOUND IN SOLN. A	Cu FOUND IN SOLN. A	Zn FOUND IN SOLN. B	Cu FOUND IN SOLN. B
	(*mg. per cc.*)	(*mg. per cc.*)	(*mg. per cc.*)	(*mg. per cc.*)
W. C. McVey, B.A.I.,	0.026	0.0045	0.093	0.0360
Washington	0.016	0.0048	0.101	0.0360
	0.020	0.0048	0.096	0.0347
	—	0.0045	—	0.0341

ANALYST	Zn FOUND IN SOLN. A	Cu FOUND IN SOLN. A	Zn FOUND IN SOLN. B	Cu FOUND IN SOLN. B
S. M. Adler, B.A.I.,	0.030	0.0043	0.096	0.0360
Kansas City	0.030	0.0043	0.077	0.0360
W. B. Fromer, B.A.I.,	0.020	0.0044	0.130	0.0354
St. Louis	0.020	0.0048	0.117	0.0350
	—	0.0048	0.119	0.0358
W. C. Owens, B.A.I.,	0.040	0.0033	0.400	0.0337
San Francisco	0.040	0.0036	0.400	0.0343
	0.030	0.0044	0.290	0.0334
F. B. Hilty, B.A.I.,	0.070	0.0065	0.093	0.0366
Chicago	—	0.0056	—	0.0367
R. H. Philbeck, B.A.I.,	0.070	0.0049	0.088	0.0368
Chicago	—	0.0045	0.096	0.0367
C. C. Gouldman, B.A.I.,	0.030	0.0053	0.060	0.0360
New York	0.030	0.0053	0.061	0.0349
E. O. Haenni	0.024	0.0038	0.102	0.0366
Food & Drug Adm.	0.024	0.0038	0.114	0.0370
Cincinnati	0.024	0.0040	—	0.0358

COMMENTS BY COLLABORATORS

W. C. McVey.—Considerable difficulty was encountered in separating the zinc from the iron and other substances so as to obtain a pure white precipitate in the final H_2S precipitation. The determination of zinc by the comparison of turbidities was not entirely satisfactory, as interfering colors were produced, making accurate comparisons with standards difficult. No especial difficulty was experienced in the determination of copper by the method specified.

W. B. Fromer.—The method for copper is easy and duplicate analyses checked very well, even with very small amounts of copper. However, the evaporation of the copper nitrate to remove excess nitric acid is very tedious in an Erlenmeyer flask and it seems that this evaporation might well be made in a crucible or beaker and the copper solution afterward transferred to the Erlenmeyer flask.

The method for zinc is very long and tedious for routine work. It was found difficult to prepare a Gooch crucible tight enough to hold the zinc sulfide precipitate even with very gentle suction. When a very thick close pad of asbestos was used complete removal of other salts by washing became doubtful. In the turbidity comparison method it was found that the turbidity of the sample increased gradually after the ferrocyanide was added while the standard tubes remained practically constant for some time. The readings reported were made almost immediately after the reagent was added. More detailed directions might well be given on this point. The analyst is far from satisfied with the results for zinc.

C. C. Gouldman.—In regard to the statement in the turbidimetric method reading: "Wash twice with H_2S water, mix, filter and precipitate in a beaker with 5 cc. HCl (1 plus 3), etc.," it is suggested that a large amount of acid, preferably

10 cc., be used to dissolve the ZnS because of the interference of the filter paper present. The methods for the determination of zinc are, in the opinion of the analyst, too long and involved for routine laboratory work.

E. O. Haenni.—No particular difficulty was encountered with any of the determinations. In general, the methods seem to be satisfactory.

CONCLUSIONS

The amount of copper in the 50 cc. aliquot of solution "A" was 0.18 mg. and was about one-third the amount (0.5 mg.) determined collaboratively last year by the same method and reported by the Referee on Gelatin. The latter reported excellent results secured by his collaborators, but the results herein reported for the smaller amount can not be considered satisfactory. Therefore between these amounts lies, apparently, the smallest amount of copper that can be determined by the tentative method with any reasonable degree of accuracy. It would appear that a supplementary colorimetric method would be desirable at this point. The uniformly good results secured in the determination of copper in solution "B" indicate that the tentative method is very reliable for quantities of copper approximating 2 mg.

The reports of collaborators indicate that both the tentative and supplementary zinc methods need further study and revision. Fairly good results have been secured by the referee and also by two or three collaborators with the tentative method in conjunction with the supplementary nephelometric method. Great care must be exercised, however, to prevent contamination by iron and other extraneous salts if this method is to yield reliable results for very small quantities of zinc. It is probable that the chief source of error in this method is the insufficient washing of the precipitate of zinc sulfide and the accompanying paper pulp. It is essential that all salts, except the exact kind and amount specified in the method, be entirely absent when the potassium zinc ferrocyanide is precipitated. For example, a constant and increasing turbidity accompanies an increasing amount of potassium chloride up to a concentration of approximately 0.1 N, or approximately 0.4 gram of potassium chloride in a 50 cc. tube. Just beyond this concentration, however, the amount of turbidity fluctuates widely, and usually decreases. Phenolphthalein can not be used as an indicator in neutralizing the hydrochloric acid as its presence definitely retards the development of the turbidity. Refiltration of very small quantities of zinc sulfide does not insure against loss because there is an insufficient amount of the sulfide formed to coat the filter paper properly. If a Gooch crucible and a very heavy pad of asbestos are used then the necessary complete washing out of dissolved salts becomes uncertain. The procedure recommended is the shaking of the zinc sulfide with a small quantity of quantitative paper pulp, filtering, gently removing the pulp from the filter, shaking with warm water, and refiltering. It is the opinion of the associate referee that the tentative gravimetric

method can be simplified and made to yield better results in the determination of quantities of zinc in excess of 2 mg., while a revised nephelometric method, or a colorimetric method employing one of the recently developed organic reagents, may be substituted for the smaller quantities.

It is recommended[1] that the tentative method for the determination of zinc be subjected to further study.

REPORT ON LEAD

By W. J. Mc CARTHY[2] (U. S. Food and Drug Administration, Cincinnati, Ohio), *Associate Referee*

Work on the colorimetric method for the determination of small quantities of lead was continued. Two samples containing 0.80 mg. and 1.60 mg. of lead respectively, with corresponding quantities of copper, iron, arsenic, and calcium and 2 grams of sugar per 50 cc. of solution, were analyzed by two collaborators with the following results.

Results expressed as milligrams of lead per 50 cc. of sample.

ANALYST	METHOD I		METHOD II		METHOD III	
	A	B	A	B	A	B
C. H. Badger	0.75	1.62	0.48	1.37	0.11	0.67
	0.69	1.41	0.34	1.27	0.18	0.69
	0.71					
W. J. McCarthy	0.70	1.38	0.71	1.27	0.79	1.28

Method I was outlined at the 1931 A.O.A.C. meeting.[3] The sample is taken through the wet ashing process, precipitated as PbS once, $PbCrO_4$ once, and then as chromium colorimetrically. Method II started with wet ashing, then followed through PbS precipitation twice, $PbCrO_4$ once, then chromium colorimetrically. Method III: wet ashing, PbS twice, $PbSO_4$ once, $PbCrO_4$ once, then chromium colorimetrically.

The analytical results obtained with Method I show a closer agreement between the analysts than those obtained with the other two methods. Taking the results as a whole (Method I) the minimum recovery is 86.25 per cent and the maximum 101.2 per cent, with an average of seven determinations 90.25 per cent. The recovery on A, containing 0.80 mg. of lead, was 89 per cent and on B, containing 1.60 mg. of lead, was 91.87 per cent.

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 58 (1933).
[2] Presented by W. J. Wichmann.
[3] *This Journal*, 15, 371 (1932).

In working with Method I the associate referee has consistently recovered 90 per cent or more of the lead known to be present. This fact, in conjunction with the tabulated results under Method I, leads to the conclusion that the method as outlined in last year's report should be adopted as a tentative method.[1]

REPORT ON FRUITS AND FRUIT PRODUCTS

By B. G. HARTMANN (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

L. H. McRoberts, Associate Referee on Soluble Solids, regrets that his study of the refractometer method for the determination of the water-soluble solids of fruit products has not progressed far enough to permit of a formal report. He has the work well in hand, however, and is assured that he will have something definite to report next year.

L. A. Salinger, Associate Referee on the Effect of H-ion Concentration on Extraction of Fruits, did not submit a report on his assignment, but collaborated with D. H. Tilden on the ash project.

B. G. Hartmann, Associate Referee on Fruit Acids, reports methods for the determination of laevo and inactive malic acids.

Salinger and Tilden's work on ash is a continuation of last year's work on methods for the determination of iron and aluminum in fruit products. The ground work for formulating methods for determining these two metals has been laid, and preliminary tests show that the principles involved are sound. It is regretted that time did not permit collaborative study. Briefly, the procedures consist of the precipitation of the metals as the phosphates, washing the phosphates with a hot 0.1 per cent ammonium acetate solution of definite H-ion concentration, precipitating the iron in the weighed phosphates with cupferron, and obtaining the aluminum by difference. An alternative method for the direct determination of aluminum is also included in the report. After the iron has been removed by cupferron, the aluminum in the filtrate is determined as the phosphate. No formal report on the subject is presented this year.

It is recommended[2] that the methods be further studied.

No report on soluble solids was given by the associate referee. See report of the Referee on Fruits and Fruit Products.

No report on ash was given by the associate referee. See report of the Referee on Fruits and Fruit Products.

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 58 (1933).
[2] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 59 (1933).

REPORT ON FRUIT ACIDS

By B. G. HARTMANN (U. S. Food and Drug Administration,
Washington, D. C.), *Associate Referee*

In accordance with the recommendations adopted last year, the study
of methods for the determination of fruit acids was continued.

Methods for laevo and inactive malic acid, which are believed to be
accurate and easily manipulated, have been formulated. The method for
laevo malic acid was published in the November issue of *The Journal*
of the Association,[1] and the modified method for the inactive acid is now
in preparation for publication.[2]

Briefly, the method for the laevo acid involves the removal of the
pectin with alcohol, the precipitation of malic acid with lead acetate, the
removal of sugars by washing the lead salts with alcohol, the removal of
tartaric acid as potassium acid tartrate, removal of tannins and citric
acid with tribasic lead acetate, and the determination of the malic acid
by polarization.

The procedure for the isolation of inactive malic acid follows the same
lines as that for laevo malic acid. The total malic acid (laevo and in-
active) is determined by oxidation with potassium permanganate in
alkaline solution, and the inactive acid is obtained as the difference be-
tween the total and the laevo malic acid.

The procedure for laevo malic acid has been used by the U. S. Food
and Drug Administration in its investigation on fruits, and the referee
is free to say that in all instances when it was applied no trouble was
experienced in any of its provisions.

The procedure for the inactive acid has not gone through the fire test,
but there is no reason to believe that it will not measure up to expecta-
tions. Certainly the experimental tests have been very gratifying.

It is recommended[3] that the resolutions adopted by the committee
last year be carried over into the next year.

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No report on effect of H-ion concentration on extraction of fruits was
given by the associate referee.

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REPORT ON CANNED FOODS

By V. B. BONNEY (U. S. Food and Drug Administration,
Washington, D. C.), *Referee*

No collaborative work was done during the past year on analytical
methods for canned foods.

[1] *This Journal*, 15, 645 (1932).
[2] *Ibid.*, 16, 277 (1933).
[3] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 59 (1933)!

P. A. Clifford, of the Food Control laboratory of the Food and Drug Administration, developed a method for measuring the turbidity of the liquor in canned peas. It is hoped that it will be published in an early number of *The Journal*.

Clifford also did considerable work on the determination of total solids in tomato products, particularly with reference to their determination by the use of the table published by W. D. Bigelow and A. E. Stevenson in National Canners Association Bulletin 21 L, as compared with the tentative method by drying given in *Methods of Analysis*. His conclusions are that the refractometric method is satisfactory as a rapid sorting method, but not suitable for inclusion as an official, or even as a tentative method. In view of the standards which have been adopted for total solids in certain concentrated tomato products, it is recommended that the methods for the determination of total solids in tomato products be given further study, with a view to the adoption of an official method, either the present tentative method or some other method which may be found to be more satisfactory. It is possible that determination of the soluble solids by means of the Abbé refractometer and of the insoluble solids by the present tentative method and adding the two results together to get the total solids, might be a better procedure than the present tentative method.

During the last few years methods have been devised in the Food and Drug Administration for the determination of the hardness of canned peas and of color of canned tomatoes, to be used in connection with the grading of these products under the Mapes amendment to the food and drugs act. Since few laboratories are equipped to make these tests, no effort was made to send out samples for collaborative study. The referee hopes that during the ensuing year it will be possible to make collaborative studies and to present them to the Association.[1]

COMMITTEES NAMED BY THE PRESIDENT

Committee to Wait Upon Dr. Woods: W. W. Skinner and G. S. Fraps.

Committee on Resolutions: J. A. LeClerc, C. C. McDonnell, H. R. Kraybill.

Committee on Auditing: B. G. Hartmann and Bailey Brown.

Committee on Nominations: R. N. Brackett, J. S. McHargue and R. C. Roark.

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 59 (1933)

DRUG SECTION

REPORT ON DRUGS

By ARTHUR E. PAUL (U. S. Food and Drug Administration, Chicago, Ill.), *Referee*

The drug section during the year gave attention to 25 topics. Although a few of the findings were negative in character, the year's work has resulted in a material advance in the drug methods of this Association. Twelve new methods were tentatively adopted.

It is recommended[1]—

(1) That the following subjects be closed:

Minute Quantities of Iodides in Mixtures
Microchemical Methods for Alkaloids
Phenolsulfonates
Sulfonal and Trional
Bromide-Bromate Methods
Ipomea, Jalap and Podophyllum
Bismuth Compounds
Emodin Bearing Drugs
Alcohol in Drugs

(2) That the following subjects be continued:

Crude Drugs
Radioactivity in Foods and Drugs
Mercurials
Santonin
Ether
Benzyl Compounds
Guaiacol
Rhubarb and Rhaponticum
Tetrachlorethylene
Calcium Gluconate
Hypophosphites
Hexylresorcinol
Microchemical Methods for Synthetics
Biological Testing
Ergot Alkaloids

(3) That the following new subjects be studied:

Small Quantities of Morphine in Sirups
Nitrites in Tablets
Ointments
Acetphenetidin in presence of Caffeine and Aspirin
Resins and Oleoresins
Gums
Pyridium
Strychnine in Tablets

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[1] For report of Subcommittee B and action of the Association, see *This Journal*, 16, 51 (1933)!

(4) That consideration be given to the subject of free salicylic acid in acetyl salicylic acid tablets. The quantitative determination of free salicylic acid in aspirin tablets was studied collaboratively some years ago, and as a result a method was adopted by this Association. Some time later when the tenth edition of the U. S. Pharmacopoeia appeared a similar method for the examination of acetylsalicylic acid itself was included. However, it is essentially qualitative only, as it merely shows whether the amount present is excessive. Calculation shows that this test is based on 0.1 per cent as a permissible maximum.

The difficulty with the determination of free salicylic acid in the presence of acetylsalicylic acid is that the latter has a strong tendency, especially in solutions, to undergo decomposition with the formation of free salicylic acid. This was, of course, given consideration in formulating the A.O.A.C. procedure. It was believed that the details were sufficiently simple and rapid to enable completion of the operation so as to involve only slight inaccuracies due to decomposition. Collaborative results bore out this assumption, and examination of many commercial samples showed only negligible amounts of free salicylic acid, when examined by the official A.O.A.C. method. Nevertheless, recent attention to various brands of aspirin tablets led to the conclusion that for small proportions of free acid the method is not so definite as might be desired and also that it might be slightly modified as to details so as to minimize the inaccuracy due to decomposition during analysis. The present method involves filtration of a water solution. It was found that any decomposition in alcohol is very slow, and it was further found that filtration of the alcoholic solution is much more rapid than that of a water solution. It is therefore preferable to filter the alcoholic solution before adding the water.

The following procedure involves only minor changes in the official details, and since there is no change in the principles of the method, its adoption without further collaborative work is recommended.

(a) *Standard salicylic acid solution.*—A recently prepared alcoholic solution containing 0.1 gram of salicylic acid per liter.

(b) *Ferric ammonium sulfate.*—Add 1 cc. of normal HCl and 2 cc. of FeNH$_4$ (SO$_4$)$_2$·12H$_2$O and dilute with H$_2$O to 100 cc.

PREPARATION OF SAMPLE

Weigh 100 tablets for average weight, triturate in a mortar to a fine powder, and keep in a tightly stoppered bottle.

DETERMINATION

In each of two colorimetric tubes mix 48 cc. of H$_2$O and 1 gram of freshly prepared Reagent (b). Shake 2.5 grams of the powdered sample with exactly 25 cc. of

alcohol and filter if necessary. Immediately add 1 cc. of the filtrate to one of the colorimetric tubes and add 1 cc. of the standard salicyclic acid solution to the other. Mix. Immediately and rapidly make color comparisons and calculate the free salicylic acid on the basis of the acetylsalicylic acid present. (If the color is too intense for satisfactory comparison, repeat the entire determination, using a smaller weight of the powdered sample.)

(5) That the following comments and recommendations, which were made in connection with associate referees' reports, constitute a part of this report:

Crude Drugs.—The associate referee reports progress in the study of crude drugs. It is recommended that the subject be continued.

Radioactivity in Foods and Drugs.—Unusual progress has been made for a number of years on this subject. Although the associate referee has not made a report on work actually done this year, his letter shows that he is preparing for distinctly advanced investigations for these highly technical determinations.

Emodin-Bearing Drugs.—The associate referee has devoted special attention to three products recognized in the U. S. Pharmacopeia, namely, fluidextract cascara, fluidextract cascara aromatic, and aloin. At the present time no methods are available for the examination of these substances. The associate referee has studied two methods and has secured results on a number of samples examined by himself and by a number of collaborators. He now recommends that these two methods be tentatively adopted. It is suggested that the methods be then retained in that status until experience has been gained as to their applicability and usefulness.

Mercurials.—The associate referee attempted to utilize some distillation methods for this determination, but found them unsatisfactory. He has evidently given no consideration to Subcommittee B's recommendation to study Rupp's formaldehyde method. It will be necessary to continue this subject next year.

Microchemical Methods for Alkaloids.—Since the beginning of the work in 1926, reports of collaborative study have been submitted yearly. Tests for 19 of the 21 alkaloids studied were considered satisfactory, and accordingly recommendations for adoption were made. It is believed that the more important alkaloids have been included in the work and that the subject may be closed for the present, as recommended last year.

Hypophosphites.—From statements made by the associate referee it seems that he has performed some work, but has not accomplished anything he thought would warrant reporting. Since this is an important subject, it is recommended that the topic be continued next year.

Santonin.—The associate referee made a careful review of the literature on the subject of artemisias assay and is devoting study to two methods which seem promising.

He has also performed laboratory work on the determination of santonin in chocolate mixtures. He investigated the Claus method and amended it by substituting benzol for ether as solvent, thereby shortening the time of extraction from 4 or 5 hours to one-half hour. The santonin recovered is apparently very pure. His results reported by the original Claus method were slightly higher than the theoretical figure. His modified method gave results closer to the truth. It is suggested that the work performed be repeated by the associate referee and that in carrying out the extraction a test be made to determine whether the extraction at the end of the half-hour period is complete so far as santonin is concerned. The associate referee's recommendations are approved.

Ether.—This difficult determination has been the subject of investigation and study for a number of years. This year, the associate referee perfected details which have yielded results remarkably close to the truth. Solids, and alcohol even in large percentages, do not interfere. Tentative adoption of his method is recommended. It was not possible for the associate referee to give consideration to the influence of essential oils or other volatile substances, and it is recommended that the subject of ether be continued for another year in order that this phase may be studied.

Benzyl Compounds.—The associate referee made considerable headway on this new topic. He devised a method for benzyl alcohol which yielded very promising collaborative results. In fact, they show a fairly uniform yield of 95 per cent of the theoretical. There is a possibility, in spite of all precautions, that the authentic benzyl alcohol may not have been absolutely pure and that the accuracy of the method is higher than indicated by the results obtained. The Associate Referee suggests that the corrective factor, 1.05, be employed. However, he also recommends that next year the determination of benzyl benzoate be studied by the same principles that were used in his benzyl alcohol method. Therefore it is recommended that action on the alcohol method be delayed until the associate referee has had an opportunity to carry on some additional work on the method. Special attention should be given to the purity of the authentic samples employed. It is hoped that the need for a corrective factor may be obviated.

Small Quantities of Iodides in Mixtures.—As attention will be devoted to it by the Associate Referee on Mineral Mixed Feeds, the associate referee's recommendation that this subject be closed is approved.

Bismuth Compounds.—A method was adopted and a recommendation to close the subject was approved in 1931.

Phenolsulfonates.—The associate referee recommends that this topic be continued. As the results reported by his collaborators, however, are quite acceptable, it does not seem necessary to continue the subject, and it is recommended that the method be adopted tentatively and the topic closed.

Sulfonal and trional.—A considerable amount of work was done on this subject, and the results reported by the associate referee are quite acceptable. The commercial demand for the products, however, has decreased greatly, in fact to such an extent that the associate referee does not recommend adoption of his methods. Since the products are recognized in the U. S. Pharmacopoeia and are still carried by wholesalers and prescribed by physicians, it would seem desirable to have methods for their determination. The associate referee and his collaborators have studied two methods, which differ only as to details. The two methods were combined into one, applicable to both sulfonal and trional by the referee.

It is recommended that this method be made tentative and that it be retained in that status. The method has been published.[1]

Guaiacol.—The associate referee made a laboratory study of some of the available methods, but found that they require further study and further modification in order to render them entirely satisfactory. His recommendation that the study of this subject be continued during the coming year is approved.

Bromide-bromate methods.—No work was done on this subject by the associate referee. However, three associate referees have studied these methods in connection with specific subjects, phenolsulfonates, guaiacol, and hexylresorcinol. It is therefore recommended that the topic be closed.

Ipomea, jalap, podophyllum.—The associate referee proposed a method for ipomea and jalap, and it is recommended that it be tentatively adopted. No work was done on podophyllum and this subject should be continued, but it is planned that the project be assumed by the Associate Referee on Gums, Resins, and Oleoresins. It is recommended that the present subject be closed.

Rhubarb and rhaponticum.—In his report the associate referee compares these two drugs from every angle,—physical, chemical, and biological. He makes suggestions for further work, and it is recommended that these be given attention.

Calcium gluconate.—The associate referee is making considerable headway in connection with the difficult problem of formulating a method for the determination of gluconate. A polarimetric method which is very promising was studied this year.

Tetrachlorethylene.—The associate referee carried on a limited amount of work, and tried out several sets of details. His report will be suggestive and helpful in connection with next year's work.

Hexylresorcinol.—The associate referee applied a new modification of the Koppeschaar procedure to effect complete bromination of hexylresorcinol by raising the initial temperature and lengthening the time for re-

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[1] *This Journal*, 16, 83 (1933).

action. The recommendations for further study of the method are approved.

Ergot alkaloids.—The associate referee made a collaborative study of a colorimetric method that requires a special apparatus. Three collaborators took part in his investigation, and their results are in remarkably close agreement among themselves and also with a careful biological test.

The extreme importance of having available a satisfactory chemical method for the examination of ergot preparations induced the associate referee to delay making a recommendation for adoption until more extensive results are obtained. He also desires to try some minor changes in the details of the method.

Microchemical methods for synthetics.—This is a new topic, which quite properly follows the extensive work which has been done on the microchemical identification of alkaloids. The associate referee devised a list of reagents, studied the reactions of four important synthetic compounds: Chinosol, benzocaine, pyridium, and cinchophen, and described and tabulated his findings. He also submitted specimens to eight collaborators for identification by his method and obtained a correct result in each instance.

His recommendation for adoption of these methods is approved and it is further recommended that the topic be continued, with the study of additional items in view.

Biological testing.—The associate referee is continuing his work on biological methods, but his investigations have not yet produced data which would warrant the preparation of a report.

No report on crude drugs was given by the associate referee.

No report on radioactivity in foods and drugs was given by the associate referee.

REPORT ON EMODIN-BEARING DRUGS

By E. O. EATON[1] (U. S. Food and Drug Administration, San Francisco, Calif.), *Associate Referee*

CASCARA SAGRADA

A modification of the method formerly proposed[2] was tried out on several samples. The results are shown in the table. The method has been published.[3]

	Extract
	gram per 100 cc.
Fluid Extract Cascara, commercial	0.55
Fluid Extract Cascara, Home Made, 1932	0.85
Fluid Extract Cascara Aromatic, Home Made	0.45
Fluid Extract Cascara Aromatic, U.S.P. (commercial)	0.30
Fluid Extract Cascara Aromatic (commercial)	0.32

COMMENTS

The essential modification is the shake out with sodium bicarbonate, which removes the traces of glycyrrhizic acid carried into the chloroform from the aromatic fluid extract. The sodium carbonate solution answers practically the same purposes as the fixed alkali formerly used, but it may have some advantage. This modification appears to give results which should check with the pysiological tests; at least they correlate well with the proposed dosage. Sufficient collaborative work has been done on this type of method.

It is recommended that the modified method be adopted as tentative.

ALOIN

No collaborative work was done on this subject this year. However, an attempt was made to learn the source of the commercial aloin in the United States. Correspondence resulted in samples being obtained from three well-known manufacturers. They all use Curaçao aloes for their crude material and have used no other for years.

Two samples obtained, of a citron-yellow color, assayed by the shake-out method 93 and 93.3 per cent by weight, and acetylized to 98.2 and 98.3 per cent of aloin extracted, respectively. Two different samples, obtained from the third manufacturer, were of a much darker color and assayed only 85 per cent by the shake-out method. These latter samples it would appear were probably never as pure as the other two, or were very old and improperly held. However, a sub-sample of one of the aloins of the lighter color was held in a colorless glass bottle, subjected to daylight for three months without apparent change in color.

Curaçao aloes is the only aloes now used commercially for the extraction of aloin. The proposed factor to convert the aloin hexylacetate to aloin is the correct one.

It is recommended[1] that the method proposed last year[2] be made tentative.

L. E. WARREN: From some experiments which I carried out several years ago in collaboration with the Pharmacological Laboratory of the Food and Drug Administration, and also working on humans, I came to the conclusion that the uncombined anthraquinones probably represented 10 or 15 per cent of the total ac-

tivity of cascara. What might be said to be a minimal dose of the material was determined by physiological tests. The uncombined anthraquinones were then removed from an equivalent amount of the preparation by an automatic extractor, and the strength of the portion remaining in the extractor was determined by similar tests. By comparing the original minimal dose with that determined in the portion from which the emodin had been removed, I judged that something like 10 per cent of the original activity had been removed. Unfortunately we cannot use the extracted material for producing physiological effects. It is inert. We must have a method which will determine it as it exists in the original material before it is extracted. I think that the question of the activity of the free emodin should not be passed upon at present. The matter should be held open and further work done.

PETER VALAER: Our laboratory is very much interested in this subject of cascara and other emodin-bearing drugs—just now, aloes in particular. The method just outlined by Mr. Warren is perhaps satisfactory for pure drugs, when we have just the one drug in a powder. We have to determine its presence among numerous other ingredients. For instance, recently we had a foreign preparation which contained about 25 different substances besides aloes, which was the principal ingredient. If we use the method described by Mr. Warren, by mere extraction we obtain a great deal of material not from aloes. This residue would be about three or four times what we would be able to get from the drug itself, and, of course, that would produce erroneous figures. We have been using methods which are colorimetric. The residue we obtain that way is recorded and taken for what it is worth, but we go one step farther and get the color by using the Bornträger reaction. Under the prescribed conditions, the color is due only to the drug in question. In 1931 I wrote an article, entitled "A Study of Emodin-Bearing Drugs." The second article had to do with aromatic cascara and the third with rhubarb. These methods were tried out in the presence of other drugs. If anyone would like to see what we have done along that line, I shall be glad to have him call at Room 422, Treasury, or write me for copies. We could not use the ordinary method unless we had a solid drug or fluidextract, which we never have.

I should like to introduce G. E. Mallory, one of my colleagues, who has done a great deal of work in the determination of aloes by the benzyl-alcohol method. It gives the total color produced by the emodin-bearing drugs.

G. E. MALLORY: This method is very short. It is proving its worth even in the presence of phenolphthalein. We take a preparation containing aloes, 4 grains to the fluid ounce, for a working standard. Take 10 cc. of sample and place in a separatory funnel, and add 5 cc. of water and 20 cc. of stronger ammonia water. Shake thoroughly and allow to stand, with occasional shaking for 2 or 3 hours—2 hours as a minimum. Then add 50 cc. of benzyl alcohol and thoroughly shake. Let stand overnight. The benzyl alcohol layer will be separated and clear by morning. We now have a nice clear red-colored benzyl alcohol solution consisting of benzyl alcohol, ammonia, ethyl alcohol, water, and extracted color. Draw this off in a 100 cc. glass-stoppered cylinder and re-extract the aqueous solution with 25 cc. of benzyl alcohol. The second extraction takes out the last portion of soluble color. This is placed in the 100 cc. cylinder and made to volume with ethyl alcohol, and its color is read in a Lovibond tintometer, the ½" cell being used. This color for Curaçao aloes and Socotrine aloes is approximately 3 on the red slide. After this color is read, evaporate off all the ammonia and make up to volume. The color is reduced to approximately 2½. Next make up the aqueous liquid that is left in a separatory funnel to 100 cc. with water and read this color in the same manner. We thus have a measure for total color produced by the emodin materials with ammonia, principally the permanency of the color that remains after all the ammonia is

driven off, and also a means for measuring the color insoluble in the solvent. Cape aloes run down to about 1.75 red color in comparison to the colors mentioned above We may get some red color due to various different dye woods used in tonics but these colors are more blackish and are not permanent. Rhubarb works fairly well by this method, but only a slight amount of work has been done. No work has been done on cascara and senna. Over 28 samples of various aloes have been analyzed in this manner and strikingly concordant results were obtained.

As a gravimetric quantitative method it is worthless because benzyl alcohol is a great solvent for many herb extractives.

Some work has also been done by hooking up the aloes with metals in order to work out an accurate gravimetric quantitative analysis. At the start I found only Curaçao aloes worked to any degree but by combining them with bismuth salts, then neutralizing and salting out I have built up to 1½ grams of ash from all three grades of aloes obtained from 25 cc. of a preparation containing 4 grains of the aloes per fluid ounce. When we can hook the aloes up with metals and take 25 cc. or even 10 cc. and consistently obtain accurate ash results we have something tangible to work towards. It will take approximately 50 or more experiments to complete this work.

A. G. MURRAY: Does the phenolphthalein interfere with the benzyl alcohol color?

G. E. MALLORY: No, that is the best part of this method, that phenolphthalein is not extracted in the least degree and gives absolutely no interference. Yellow phenolphthalein in which emodin-bearing materials are formed in the process of manufacture and to which the added activity of the phenolphthalein is due will naturally show that measure of color due to that portion present.

No report on mercurials was given by the associate referee.

REPORT ON MICROCHEMICAL METHODS
FOR ALKALOIDS

By C. K. GLYCART[1] (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

As recommended last year, the microchemical study of alkaloids included nicotine, lobeline, and sparteine. In the preliminary work no suitable test for lobeline was found. The directions for the tests and control specimens and samples for identification were sent to the collaborators. The controls consisted of sparteine sulfate, and a 1:100 solution of nicotine sulfate prepared in the laboratory by steam distillation from a commercial solution of nicotine sulfate.

Sample No. 1 for identification consisted of a commercial spraying compound diluted to contain approximately 1:100 nicotine sulfate in solution, and No. 2 was a 1:100 sparteine sulfate solution.

The method has been published.[2]

Consistent results: No. 1, nicotine; No. 2, sparteine, were obtained by

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the following collaborators: H. McCausland, Abbott Laboratories, North Chicago; Charles C. Fulton, Treasury Department, Minneapolis; E. O. Eaton, U. S. Food & Drug Adm., San Francisco; Jonas Carol, U. S. Food & Drug Adm., Chicago; Irwin S. Shupe, U. S. Food & Drug Adm., Chicago.

SUMMARY

Since the beginning of the work in 1926, 21 of the more important alkaloids have been studied. Microchemical tests and descriptions have been recommended for the following 19 alkaloids: Aconitine, arecoline, atropine, brucine, caffeine, cinchonine, cinchonidine, cocaine, codeine, ephedrine, heroine, morphine, nicotine, pilocarpine, quinine, quinidine, sparteine, strychnine, and yohimbine.

No suitable tests were obtained for lobeline and physostigmine. Millon's reagent[1] (mercuric nitrate solution) is unreliable for alkaloidal testing as a crystalline precipitate forms immediately on dilution. Silver nitrate[1] solution is also misleading for the reason that crystals of silver sulfate are formed in the presence of dilute solutions of sulfates of alkaloids.

Stephenson's[2] book, "Some Microchemical Tests for Alkaloids," was considered of especial value. References to photomicrographic plates were purposely omitted, but experience of the microscopist with the appearance of the characteristic crystals obtained with the control specimens and specified reagents was considered important.

RECOMMENDATIONS[3]

It is recommended—

(1) That the tests and descriptions for nicotine and sparteine be adopted as tentative.

(2) That the subject be closed for the present.

No report on hypophosphites was given by the associate referee.

REPORT ON SANTONIN

By H. M. BURLAGE (School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina), *Associate Referee.*

During the past year no collaborative studies were conducted. The associate referee spent most of his time in examining proposed methods to determine their suitability. Efforts were directed along the following lines: (1) study of the methods suggested for the determination of santonin in the artemisias and (2) examination of recently proposed methods for the estimation of santonin in chocolate (fatty) mixtures.

[1] *Am. J. Pharm.*, 104, No. 4 (1932).
[2] J. B. Lippincott (1921).
[3] For report of Subcommittee B and action of the Association, see *This Journal*, 16, 52 (1933).

In a dissertation presented as a partial fulfilment of the requirements for a graduate degree Smith with Burlage[1] studied a number of the published methods for this assay and as a result concluded that the methods of Feldhoff[2] and Eder and Schneiter[3] are the most promising. Others were those of Janot and Mouton[4] and Herndelhoffer.[5] The authors also propose a colorimetric procedure involving a modification of the Feldhoff method and an "alcohol method," which seem to be satisfactory. These methods are being given further consideration by the associate referee. Quite recently Coutts[6] suggested the use of benzene as the primary solvent and the removal of interfering substances by the use of a solution of sodium carbonate. These methods, however, have been found to be unsatisfactory. Fernandez and Sociàs[7] recently proposed a method whereby santonin is extracted from the crude drug in a manner similar to that proposed by Feldhoff and freed finally from all impurities by conversion of the santonin into a derivative with 2:4 dinitrophenylhydrazine. This method will be given consideration in these laboratories very shortly. Work of a definite character should be accomplished this year and a specific recommendation concerning this difficult assay be made at the next meeting.

Very recently Claus[8] proposed a method for the determination of santonin in troches containing chocolate. This method follows:

Extract the material 4–5 hours with ether in a Soxhlet extractor. After filtering, distil off the ether and add ½ gram of paraffine. Add 50 grams of alcohol (80%) and boil for ½ hour under a reflux. After cooling, filter through a filter wetted with alcohol (80%), receiving the filtrate in a separatory funnel. Boil the residue with 15 grams of alcohol (80%), cool, and pour through the same filter. Shake the filtrate in the separatory funnel with 15 grams of petroleum ether (b.p. 40–70° C.). Draw off the alcohol layer into a flask (in the following experiments a tared flask was used) and wash the petroleum ether layer with 15 grams of alcohol (80 per cent). Add this alcohol washing to the portion in the flask. Boil the alcohol solution for a short time to expel the petroleum ether (in the following experiments the solvent was carefully evaporated and the residue weighed to constant weight at 105° C.) and when cooled neutralize with 0.1 N NaOH, add 10 cc. of the standard alkali in excess, and boil the solution under a reflux for 20 minutes. Titrate the excess of alkali with 0.1 N acid, using phenolphthalein as an indicator. A blank test is run in order to overcome error due to any action of the container. 1 cc. of 0.1 N NaOH = 0.0246 gram of santonin.

Degner's[9] method recently proposed is quite similar to the method outlined above.

In order to test this method a sample was prepared corresponding in

[1] *This Journal*, 15, 491 (1932).
[2] *Pharm. Z.*, 70, 661 (1925); *C.A.*, 19, 2387 (1925).
[3] *Schweiz. Apoth. Zeit.*, 63, 405 (1925); Year Book Am. Pharm. Assoc., 14, 381 (1925); 15, 393 (1926); Chemist and Duggist, 103, 423 (1925).
[4] *Bull. sci. pharmacol.*, 37, 337 (1930); *C.A.*, 24, 4119 (1930).
[5] *Mikrochemie*, 5, 21 (1927); *C.A.*, 22, 971 (1928).
[6] *Pharm. J.*, 129, 262 (1932).
[7] *J. pharm. chim.*, 124, 49 (1932); *C.A.*, 26, 4414 (1932).
[8] *Pharm. Weekblad.*, 68, 414 (1931); *J. Pharm. Belg.*, 13, 427 (1931); *C.A.*, 25, 3432 (1931).
[9] *Pharm. Press*, (1931), 115; *Pharm. J.*, 129, 135 (1932); *B.C.A.*, (B-1932), 482.

composition to *Tabellae Santonini* of the National Formulary V. (p. 234) with a santonin content of 9.98 per cent.

Results (Claus method)

Sample	Gravimetric	Volumetric	Deviation
	per cent	per cent	per cent
I	10.74	10.57	+0.59
II	10.88	10.54	+0.56

This method, which employs ether as the primary solvent is time-consuming (4–5 hours for extraction). The residues obtained even after purification are colored to such an extent that it was difficult to obtain definite end-points. Significant quantities of fatty substances and fatty acids are removed by the extended extraction as gravimetric and volumetric results are appreciably high; large quantities of alkali were also required to neutralize the alcoholic solution before digestion with the standard alkali.

Accordingly the Claus method was modified by using benzene as the primary solvent and extracting in a Soxhlet extractor for ½ hour. The time of extraction was thus reduced appreciably.

Results obtained with the modified Claus method

Sample	Gravimetric	Volumetric	Deviation
I	10.23 per cent	10.03 per cent	+0.05 per cent
II	10.54	10.05	+0.07

The modified method requires much less time, yields residues which are almost colorless, and requires less alkali to neutralize the alcoholic solution, indicating a lesser degree of extraction of undesirable fatty substances. Satisfactory end points were obtained upon titration.

It is recommended[1]—

(1) That the investigations on the assay of the artemisias be continued for another year.

(2) That the proposed modification of the Claus method for the assay of santonin in chocolate mixtures be subjected to a collaborative study.

REPORT ON ETHER

By W. F. KUNKE[2] (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

Last year it was recommended[3] that the subject of ether be continued. Accordingly, investigational work was done to develop a quantitative method for ether which would be applicable to ether and ether-alcohol or ether-alcohol-water solutions, containing, within very wide limits, varying quantities of ether, alcohol, or water.

[1] For report of Subcommittee B and action of the Association, see *This Journal,* 16, 52 (1933).
[2] Presented by L. E. Warren.
[3] *This Journal,* 15, 49 (1932).

After considerable experimental study a simple, practical and reasonably accurate method was devised by the associate referee. The procedure is based upon a quantitative study, (1) of the reaction of ether with sulfuric acid-potassium dichromate solution of varying sulfuric acid and dichromate concentrations, (2) of the vaporization of ether from a comparatively large quantity of alcohol and/or water, and (3) of the separation of ether vapor from alcohol vapor by the absorption of the latter with sulfuric acid (1+1) during aspiration with air.

The proposed method was subjected to collaborative study. The results obtained by the collaborators are included in this report.

REVIEW OF THE LITERATURE

The U. S. Pharmacopeia gives qualitative chemical and physical tests for identity and purity of ether, but no method of assay. A review of the literature did not reveal an accurate method specifically developed for the quantitative separation and determination of ether in a sample containing a comparatively small quantity of ether in aqueous or hydroalcoholic solution, which simulates a distillate obtained from a medicinal preparation.

Various procedures for the estimation of ether in medicinal preparations, motor fuels, or combustible vapors have been published.

Haggard[1] oxidizes the ether by iodine pentoxide at 200° C. with liberation of iodine, which is caught in a potassium iodide solution. Connerande[2] passes the vapors of alcohol and ether over activated charcoal and converts into ethyl iodide by boiling the charcoal under pressure in fuming hydriodic acid. The ethyl iodide is driven off, absorbed in silver nitrate solution and the silver iodide formed is weighed. Dommer[3] and Bibel[4] base their methods upon the viscosity resistance of ether in air when drawn through capillary tubes. Masson and McEwan[5], Formanek[6], Meyer[7], and Wratschke[8] extract the ether from ether-alcohol-water solution with petroleum ether. The increase in volume of petroleum ether, after corrections have been made for the partition of ether and alcohol between the two layers, indicates the quantity of ether present.

Szeberenyi[9] determines a small quantity (1–4 per cent) of alcohol in ether, and ether in ether-alcohol-water solution containing not more than 2 per cent alcohol, by the use of different concentrations of dichromate and sulfuric acid which have a selective action. Corrections are made for the dichromate consumed by the ether, and for the incomplete oxidation of the alcohol.

[1] *J. Biol. Chem.*, **55**, 131–43 (1923).
[2] Chimie & industrie Special No. 696–9 (March, 1931).
[3] *Chem. Ztg.*, **51**, 413 (1927).
[4] *Zentr. Gewerbehyg. Unfallverhüt.*, **14**, 291 (1927).
[5] *J. Soc. Chem. Ind.*, **40**, 29 T (1921).
[6] *Chem. Ztg.*, **52**, 325 (1928).
[7] *Pharm. Ztg.*, **75**, 92 (1930).
[8] *Ibid.*, **75**, 319 (1930).
[9] *Z. anal. Chem.*, **54**, 409 (1915).

Somogyi[1] determines small quantities of alcohol and ether vapors in presence of one another by passing the vapors first through a tube containing sulfuric acid (1+3), which absorbs the alcohol and not the ether. Then the ether vapors are passed through a solution of 50 cc. each of normal potassium dichromate and sulfuric acid, which immediately oxidizes the ether to acetic acid. The excess dichromate is determined iodometrically, and from the quantity of dichromate consumed the ether is calculated.

These methods were not studied experimentally because they appeared to have very narrow limits of application. In some cases, the necessary apparatus was too complicated and expensive, and in others, the procedure was not sufficiently accurate, although practical.

PREVIOUS A. O. A. C. WORK

The associate referee[2] for 1926 made no report but considered that the Somogyi method was worthy of study. During 1927 the associate referee[3] made a study of the Somogyi method and after repeated trials concluded that it is not dependable for mixtures such as may be obtained by distillation from medicinal preparations, although it may give satisfactory results for anhydrous alcohol and ether; consequently, a modification of the method was tried. The alcohol vapor absorption tube was kept at 50°C. and the sulfuric acid-dichromate solution at the temperature of ice water.

In three determinations of ether in ether-alcohol-water (approximately 35+40+25) solution, the results obtained were 97.4, 101.0 and 102.0 per cent recovery. The largest sample contained 0.2072 gram of ether and 0.2194 gram of alcohol.

The associate referee[4] for 1928 eliminated the constant temperature bath (50°C.) and for the alcohol vapor absorption solution used sulfuric acid (1+2) instead of sulfuric acid (1+3). A range from 97.7–102.1 per cent recovery was reported in four determinations of anhydrous ether samples, the variation being from 69.6–129.3 mg. per sample. From 94.7–99.0 per cent recovery was obtained in six determinations of ether in ether-alcohol-water solutions, containing from 80.9–126.0 mg. of ether and from 85.7–166.7 mg. of alcohol per sample.

During 1929 the time available was devoted mainly to devising a satisfactory procedure for preparing aqueous and hydroalcoholic solutions of ether, which could be used for collaborative work. No reports were made in 1930 and 1931. No collaborative work on ether was done previous to 1932.

Somogyi, as did the previous associate referee, reported good results

[1] *Z. angew. Chem.*, 39, 280 (1926)
[2] *This Journal*, 10, 383 (1927).
[3] *Ibid.*, 11, 860 (1928).
[4] *Ibid.*, 13, 288 (1929).

considering the extreme care with which such a volatile substance as ether must be handled. Somogyi made determinations only on absolute ether or absolute ether-anhydrous alcohol samples and the associate referees for 1927 and 1928 also made reports on ether-alcohol-water solutions containing high percentages of ether and alcohol. However, such samples do not simulate solutions of ether most likely to be met with in a drug laboratory.

PRELIMINARY STUDY OF REACTION OF ETHER WITH ACID-DICHROMATE

Obviously, in order to work out an accurate method, it is necessary to establish carefully the most favorable and correct conditions, such as duration of reaction, concentration of reagent, temperature, etc. Theoretically, the reaction between ether and potassium dichromate and sulfuric acid proceeds according to the following equation:

$$3C_2H_5OC_2H_5 + 4K_2Cr_2O_7 + 16H_2SO_4$$
$$= 6CH_3COOH + 4K_2Cr_2(SO_4)_4 + 19H_2O.$$
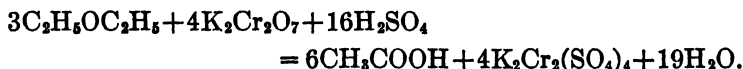
Likewise 3 molecules of alcohol are oxidized to 3 molecules of acetic acid. Under suitable conditions most organic substances may be oxidized quantitatively to carbon dioxide and water with strong oxidizing agents but with potassium dichromate in a strongly acid solution complete oxidation does not take place.

It was found that under the conditions of the proposed method, acetic acid (0.260 equivalent to 0.163 gram of ether) does not react with 0.5 N acid-dichromate solution. However, after a long period of contact (4 days) the dichromate in acid solution was partially reduced by the acetic acid. The dichromate consumed calculated to ether was equivalent to 0.37 per cent on the basis of a 0.1753 gram sample of ether.

In these experiments there was no rise in temperature of the reaction mixture. When 0.1753 gram of ether in 5 cc. of aqueous solution is allowed to react with 50 cc. of 0.5 N sulfuric acid-potassium dichromate solution, the temperature rises to a maximum of 37°C. In order to study this temperature influence on the possible oxidation of acetic acid, 0.2 cc. of glacial acetic acid was added to one of duplicate determinations of ether. The percentage of ether found differed only 0.1 per cent.

These experiments show that under the conditions of the proposed method, added acetic acid is not oxidized appreciably.

With a view to learning what the optimum conditions are as regards acid concentration and length of the reaction period, experiments given in Table 1 were made.

The ether sample was added directly to the acid-dichromate in a flask (without aspiration) and was left to stand at room temperature for the time indicated. The excess acid-dichromate was determined iodometric-

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TABLE 1

*Determinations of ether under varying conditions.**

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EXP. NO.	ETHER†	POTASSIUM DICHROMATE NORMAL	SULFURIC ACID 95%	REACTION PERIOD	ETHER FOUND
	gram	*cc.*	*cc.*	*hours*	*% by weight*
1	0.3525	50	50	1/2	99.8
2	0.3525	50	50	1/2	100.0
3	0.3525	50	50	1/12	91.5
4	0.3525	50	50	1/12	92.0
5	0.2353	50	25	16	93.0
6	0.2353	30	25	1/2	90.3
7	0.2353	30	25	1/2	86.7
8	0.3525	50	25	1/2	26.2
9	0.3525	50	15	1/2	1.1
10	0.3525	50	5	26	1.3
		0.05 N			
11	1.1753	30	5	1-1/2	0.05
12	0.0352	100	5	27	0.60

* Aspiration was not used.

† In aqueous solution prepared as directed under "Preparation of Sample."
10 cc. aliquots in Exp. Nos. 1–4 and 8–10, inclusive.
5 cc. aliquots in Exp. Nos. 5–6–7.

ally, and from the amount consumed the ether was calculated. One cc. of N acid-dichromate solution = 9.256 mg. of ether.

The results show clearly that an equal volume of both normal potassium dichromate solution and sulfuric acid is the best oxidizing reagent and that a reaction period of 30 minutes is sufficient.

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APPARATUS USED

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Aspiration with air (apparatus as illustrated, with all containers and solutions at room temperature) was used in the work reported this year. No heat was used, because it was desired to work with ether samples containing comparatively large quantities of alcohol and/or water.

The aspiration train is practical and inexpensive, and it can be made in the laboratory. A detailed description of the apparatus is given later. Two cylinders (C and D) were used for the alcohol vapor absorption solution, because it is believed that two cylinders are more efficient and practical than one container having a column height of absorption solution equal to that of the two cylinders. For a similar reason, the three cylinders (E, F and G) were used for the 0.5 N sulfuric acid-potassium dichromate solution.

The 100 cc. of 0.5 N sulfuric acid-dichromate solution in wash bottle A washed the air free from any vapor, which if present, would reduce the

0.5 *N* sulfuric acid-dichromate solution in cylinders E, F and G, and give too high results.

Bottle H was introduced as a safety reservoir. Bottle I contained concentrated sulfuric acid to dry the air before entering the soda-lime tube, J. The soda-lime prevents the acetic acid formed by the oxidation of ether, from passing into the pump. The operation of the aspiration train is described later.

ABSORPTION SOLUTION FOR ALCOHOL

In order to determine quantitatively the ether in a sample containing alcohol, the ether must first be separated from the alcohol, because alcohol and ether are both oxidized by potassium dichromate and sulfuric acid to acetic acid. Szeberenyi[1] determines ether in ether-alcohol-water solution without the separation of the ether, but the results are not so accurate as could be desired.

Somogyi[2] uses sulfuric acid (1+3) as the absorption solution for the alcohol vapor and reported that not more than 0.20 gram of alcohol vapor is absorbed by 100 cc. of the sulfuric acid (1+3). He found that if a larger quantity of alcohol is present in the sample, a correspondingly larger volume of sulfuric acid (1+3) must be used, since the attempts to vary the sulfuric acid concentration according to the quantity of alcohol introduced no longer gave accurate results. The associate referees for 1927–8 used a maximum of 0.2194 gram of alcohol per ether sample.

These workers used heat to vaporize the sample, consequently as the air used for aspiration passed into the alcohol absorption solution it contained a high percentage of alcohol vapor, and any appreciable quantity of alcohol, in excess of 0.20 gram, in the sample was not quantitatively absorbed. For the determinations reported this year sulfuric acid (1+1) was used as the alcohol-absorption solution, and all solutions and containers were kept at room temperature. This procedure proved to be very efficient.

ABSORPTION CAPACITY OF SULFURIC ACID (1+1)

Aspiration with air for 3 hours was carried out in each of four experiments for which were used 20 cc. each of 2, 5, 10, and 20 per cent alcohol corresponding to 0.3145, 0.786, 1.572, and 3.144 grams, respectively, of alcohol in cylinder B, 35 cc. of the sulfuric acid in each of the two cylinders C and D, and 40 cc. of 0.5 *N* sulfuric acid-potassium dichromate in each of the cylinders E and F. After the aspiration period in each case the titration of the dichromate solution showed that no alcohol vapor had passed through the sulfuric acid and reduced the dichromate solution. Aspiration proceeded at a rapid rate—approximately 150 bubbles per minute.

[1] *Z. anal. Chem.*, **54**, 409 (1915).
[2] *Loc. cit.*

In another experiment (Sample H1, Table 3), 30 cc. of ether-alcohol-water solution containing 35.06 mg. of ether and 10 grams of alcohol was aspirated for $3\frac{1}{2}$ hours and all the alcohol vapor which passed over with the air was absorbed by the 70 cc. of sulfuric acid $(1+1)$—35 cc. in each of two cylinders. The sulfuric acid absorbed 3.3 grams of alcohol—the difference between the alcohol content of the sample liquid before and after aspiration. More than 10 grams of alcohol per sample (25 cc. of 50 per cent by volume of alcohol) was not tried.

Theoretically, 10 grams of alcohol, if it all passed over into an excess of acid-dichromate solution, would reduce 2160 cc. of 0.5 N sulfuric acid-dichromate solution.

Other experiments were made (as shown in tables later) which indicate that sulfuric acid $(1+1)$ is an efficient absorption solution for alcohol vapor.

ETHER SAMPLE USED

According to U.S.P. X., 1 cc. of ether is soluble in about 12 cc. of water at 25°C. Absolute ether was used in making up the samples of ether in aqueous solution for the work reported this year. These solutions were prepared as directed under "Preparation of Sample." Usually 5 cc. of ether, accurately weighed by difference, was dissolved in sufficient water to make 100 cc. In five weighings the range in weights was from 3.504 to 3.507 grams and the variation from the average was -0.04 and $+0.04$ per cent. At the time of weighing the room temperature varied from 25° to 26° C.

In the experiments where a separation of the ether from the alcohol was made by absorption of the alcohol vapor by sulfuric acid $(1+1)$ and aspiration, the ether-water solution was introduced into cylinder B, which contained the given quantity of alcohol.

This scheme permitted comparative determinations of ether to be made on aliquots of the same sample and the use, first, of the direct acid-dichromate method (without aspiration); second, aspiration of the ether vapor from the ether-water solution through the sulfuric acid $(1+1)$ into the acid-dichromate solution; and third, separation of the ether vapor from the alcohol vapor, by absorption of the latter by sulfuric acid $(1+1)$ and subsequent oxidation of the ether by the acid-dichromate solution.

DETERMINATIONS OF ETHER, WITH AND WITHOUT ASPIRATION

For the experiments without aspiration a 250 cc. ground-glass stoppered flask was used, and for those with aspiration the illustrated apparatus was used. The determinations were made by the proposed method, except that in some cases the aspiration period, the reaction time, or the quantity of ether was varied.

Table 2 gives the results obtained and also the quantity (cc.) of normal

acid-dichromate solution consumed in each cylinder. In the determinations using the direct acid-dichromate method (no aspiration) 25 cc. of each normal potassium dichromate solution and sulfuric acid (95 per cent) was used, after cooling to room temperature or 50 cc. of 0.5 *N* sulfuric acid-potassium dichromate solution, Reagent (c). In the determinations using aspiration the quantities of reagents as directed in the proposed method were used, except for samples Nos. A1 and C1, when 40 cc. of 0.5 *N* acid-dichromate solution was used in each of the three cylinders. Aspirations were made at a rapid rate—approximately 150 bubbles per minute.

TABLE 2

Comparative results of determinations of ether in aqueous solution.

SAMPLE*		PERIOD		NORMAL ACID-DICHROMATE SOLUTION CONSUMED			ETHER FOUND
		ASPIRATION	REACTION	CYLINDER			
NO.	ETHER			E	F	G	
	gram	hours	hours	cc.	cc.	cc.	(% by weight)
A1	0.3460	5	—	19.95	12.06	0.31	86.3
C1	0.3454	2½	—	20.01	15.71	0.67	98.1
C4	0.1727	—	1⅔	—	—	—	99.6
C5	0.1727	—	1⅔	—	—	—	97.6
C6	0.1727	2	—	17.25	0.79	0.00	96.7
C7	0.1727	1¾	—	16.43	0.90	0.17	93.9
C8	0.1727	—	¾	—	—	—	97.4
C9	0.1727	—	¾	—	—	—	97.4
D1	0.1727	—	2	—	—	—	101.7
D2	0.1727	—	2	—	—	—	102.0
D3	0.1727	—	1	—	—	—	99.8
D4	0.1727	4	—	18.05	1.03	0.00	100.6
D5	0.1727	2½	—	16.14	0.32	0.00	86.9
D6	0.1727	6	—	18.01	1.00	0.00	100.3
D7	0.1727	4	—	17.01	0.35	0.00	91.6
D8	0.1727	—	½	—	—	—	96.9
D9	0.1727	—	1	—	—	—	99.8
D10	0.1727	5	—	17.65	0.93	0.08	98.5
E1	0.1752	5	—	19.58		0.00	104.0
E2	0.1752	—	1	—	—	—	104.3
E3	0.1752	—	⅔	—	—	—	103.0
I1	0.0035	—	2	—	—	—	102.0
I3	0.0035	—	2	—	—	—	99.8

* C1, C4, etc., each represents a 5 cc. aliquot of the 100 cc. ether-water solution, which contained the given quantity of ether per 5 cc. of solution when prepared.

The results obtained (Table 2) for samples A1 and C1 (86.3 and 98.1 per cent) indicate that the ether content (0.3460 gram) was too large to be completely carried over consistently as a vapor into the oxidation reagent within 5 hours of aspiration. The ether found in C6, C7, D5 and D7 indicates that aspiration for 1¾ to 4 hours is not long enough. (Com-

pare with D4, D6, D10 and E1). The results obtained for D6, D10 and E1 indicate that 5 or 6 hours of aspiration should be used.

ETHER IN AQUEOUS AND HYDRO-ALCOHOLIC SOLUTIONS

Table 3 gives the results of determinations of ether, (1) in aqueous solutions directly by treatment with an excess of acid-dichromate in a flask at room temperature (without aspiration), and (2) in hydro-alcoholic solutions and the use of aspiration with air to carry the ether vapor through the sulfuric acid (1+1), which absorbs the accompanying alcohol vapor, into the oxidation reagent.

TABLE 3

Comparative results of determinations of ether in aqueous and hydro-alcoholic solutions.

SAMPLE*		PERIOD		NORMAL ACID DICHROMATE SOLUTION CONSUMED			ALCOHOL†		ETHER FOUND
NO.	ETHER	ASPIRATION	REACTION	CYLINDER					
				B	F	G			
	gram	*hours*	*hours*	*cc.*	*cc.*	*cc.*	*cc.*	*% by vol.*	*% by wt.*
F1	0.1753	—	1	—	—	—	—	—	102.0
F2	0.1753	5	—	18.62	0.68	0.00	20	10	101.9
F3	0.1753	—	1	—	—	—	—	—	103.3
F4	0.1753	—	3	—	—	—	—	—	100.4
F5	0.1753	6	—	18.50	0.55	0.00	20	20	100.6
F6	0.1753	—	½	—	—	—	—	—	100.1
F7	0.1753	5	—	18.14	0.53	0.00	20	50	98.5
F8	0.1753	—	1	—	—	—	—	—	97.2
F9	0.1753	—	3	—	—	—	—	—	98.0
G1	0.1751	3½	—	19.04	0.65	0.00	20	20	103.9
G2	0.1751	9	—	18.78	0.80	0.00	20	20	103.5
G3	0.1751	6	—	18.26	1.10	0.00	20	20	102.4
H1	0.0035	3½	—	3.87	0.06	0.00	25	50	103.6
H2	0.0035	—	2	—	—	—	—	—	99.5
H3	0.0035	—	2	—	—	—	—	—	103.4
H5	0.0035	4	—	3.94	0.06	0.00	20	50	105.6
H6	0.0035	—	2	—	—	—	—	—	100.3

* F1, F2, etc., each represents a 5 cc. aliquot of the same 100 cc. ether-water solution, which contained the given quantity of ether per 5 cc. of solution, when prepared.

† Quantity of alcohol in cylinder B to which the 5 cc. aliquot of ether-water solution was added.

The results of the experiments (Table 3) in which alcohol was added to the ether sample show that it is fairly safe to have as much as the equivalent of 25 cc. of 50 per cent alcohol in an ether sample, if the total volume is not less than 25 cc. For sample F7, to which 20 cc. of 50 per cent alcohol had been added, the recovery was 98.5 per cent, which compares very favorably with results obtained for F6, F8 and F9, to which no

alcohol had been added, and the determination was made directly in a flask, without aspiration. It is true that the ether found in the case of H1 and H5 is somewhat high, but the results appear to be reasonably good when it is taken into consideration that, first, the quantity of ether in each sample was only 35 mg. and, second, a variation of 0.05 cc. (1 drop) of 0.05 N sodium thiosulfate solution (17.3 and 17.25 cc.) makes a difference of 1.3 per cent on the basis of a sample containing 35 mg. of ether. It would seem that if any alcohol vapor passes through the sulfuric acid in cylinders C and D, 3 to 6 hours longer aspiration would give appreciably higher results. (Compare results for G1 with G2 and G3.)

PROPOSED METHOD

REAGENTS

(a) *Sulfuric acid.*—(1+1). Carefully add concentrated sulfuric acid to an equal volume of water and cool to room temperature.

(b) *Potassium dichromate solution.*—1 N. Dissolve 49.0330 grams of pure potassium dichromate (or corresponding quantity of known purity) in sufficient water to make 1 liter.

(c) *Sulfuric acid-potassium dichromate solution.*—0.5 N. Carefully add 500 cc. of concentrated sulfuric acid to 500 cc. of 1 N potassium dichromate solution (accurately measured in a volumetric flask), and cool to room temperature. Use two 1 liter flasks for mixing and cooling. Transfer into a 1 liter volumetric flask, add sulfuric acid (1+1) washings and fill to mark with the sulfuric acid. Mix thoroughly.

Standardize against 0.05 N sodium thiosulfate solution as follows:

Pipet exactly 25 cc. of Reagent (c) into a 250 cc. ground-glass stoppered volumetric flask and dilute to mark with water after cooling to room temperature. Mix thoroly. Pipet a 50 cc. aliquot into a 500 cc. ground-glass stoppered flask; add 100 cc. of water, 10 cc. of sulfuric acid, and 10 cc. of potassium iodide solution—25 per cent. Stopper flask and allow to stand from 3 to 5 minutes. Add 150–200 cc. of water and then titrate with 0.05 N sodium thiosulfate, using starch solution as indicator.

(d) *Sodium thiosulfate solution.*—0.05 N.

(e) *Potassium iodide solution.*—25 per cent. Freshly prepared.

(f) *Starch solution.*—0.05 per cent. Freshly prepared.

APPARATUS

Set up the apparatus as illustrated. Beginning at the air intake end of the aspiration train, use a 400 cc. bottle as wash bottle (A), six 50 cc. graduated cylinders, having an inside diameter of 1.5 cm. and a height of 32–35 cm. (B-C-D-E-F-G), a 500 cc. bottle as safety reservoir (H), and a 400 cc. bottle as wash bottle (I), which is supplied with a soda-lime tube. Supply each container with a closely fitting rubber stopper and vapor carrying tubes. The intake tube should extend almost to the bottom, and the outlet tube, 1 cm. below the rubber stopper. Use heavy-walled glass tube having an outside diameter of 5 mm. Draw the outlets of all vapor carrying tubes down to small openings. Use heavy-walled rubber tubing for connections and between cylinders expose only 0.5–1 cm. to the vapors.

PREPARATION OF SAMPLE

Carefully weigh a 100 cc. ground-glass stoppered volumetric flask containing 65–70 cc. of water. Pipet 5 cc. of ether, holding the pipet just above the water in the flask, and as the level of the water is raised by the draining of the ether into the

flask, raise the pipet correspondingly to avoid contact with the water. Immediately stopper the flask and weigh. The difference in weight is the weight of ether. Carefully and gently swirl the liquid in the flask until the ether is dissolved and then fill to mark with water. Stopper flask and thoroly mix.

If the unknown ether sample is an alcoholic or hydroalcoholic solution, prepare a solution by dilution with water, which meets the requirements given under "Determination."

PRELIMINARY CHARGING OF APPARATUS

Transfer about 100 cc. of 0.5 *N* sulfuric acid-potassium dichromate solution into wash bottle A, and 35 cc. of the sulfuric acid solution Reagent (a), into each cylinder, C and D. (Use a funnel with a long stem to avoid wetting upper portion of container with reagent.) Pipet 40 cc., 25 cc., 25 cc. of 0.5 *N* sulfuric acid-potassium dichromate solution, Reagent (c), into cylinders E, F and G, respectively. (Avoid unnecessary wetting of the outside of the stem of the pipet and do not touch the inside of cylinder with the wetted stem of the pipet while draining.) Bottle H remains empty. Transfer about 50 cc. of concentrated sulfuric acid into bottle I and fill tube J with an appropriate quantity of soda-lime, layered on bottom and top with cotton. Stopper tightly all containers except cylinder B. Leave all rubber tubing connections between cylinders and glass stopcocks K and L open.

DETERMINATIONS

Pipet an aliquot of the sample containing 35–200 mg. of ether in aqueous solution or hydro-alcoholic solution, containing not more than 5 grams of alcohol, into cylinder B containing sufficient water to make a total volume of 25 cc. Hold the pipet just above the top level of the liquid in the cylinder, and as the liquid is raised by the draining of the sample, raise the pipet correspondingly so as to avoid contact with the liquid.

If the sample is known not to contain alcohol or other substances which will be oxidized by Reagent (c), pipet an aliquot as directed above into a 250 cc. ground-glass stoppered flask containing 50 cc. of Reagent (c). Stopper flask, swirl gently, and allow to stand for 1 hour. Titrate the excess acid dichromate and calculate as directed below.

Stopper tightly and immediately connect with cylinder C and wash bottle A. Connect the suction pump at M (Fig. 1), and with stopcock L about half open start the pump. With bottle H and cylinder G connected, gradually close stopcock K until a slow current of bubbles passes through the reagent in cylinder F and connect cylinder E. Repeat until cylinder B, which contains the sample, is connected. Make
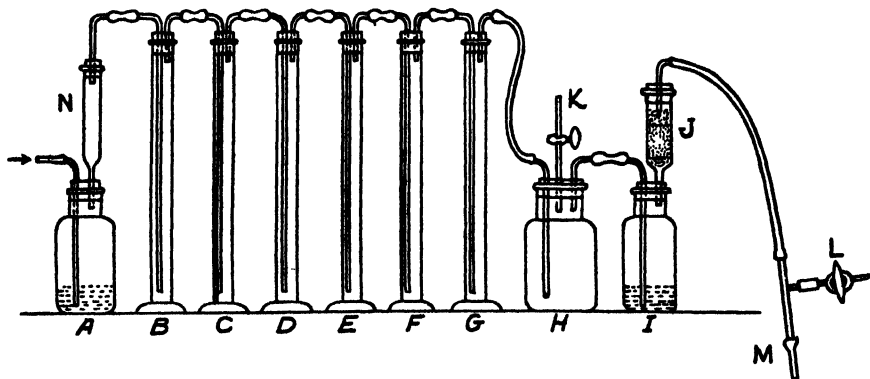
FIG 1. APPARATUS FOR THE DETERMINATION OF ETHER.

TABLE 4
Collaborative results.

COLLABORATOR	SAMPLE*		PERIOD		N ACID DICHROMATE SOLUTION CONSUMED			ALCOHOL†		ETHER FOUND
					CYLINDER					
	NO.	ETHER	ASPIRATION	REACTION	E	F	G	cc.	% by volume	% by weight
		gram	hours	hours	cc.	cc.	cc.			
J. Carol	J1	0.1753	5	—	18.32	0.96	0.00	20	10	101.8
	J2	0.1753	—	1	—	—	—	—	—	100.9
	J3	0.1753	—	1	—	—	—	—	—	101.7
Henry R. Bond	J4	0.1753	5	—	18.48	0.63	0.00	20	10	100.8
	J5	0.1753	—	1	—	—	—	—	—	100.4
	J6	0.1753	—	1	—	—	—	—	—	99.8
J. Carol	J7	0.1753	5	—	17.77	0.78	0.00	20	10	98.0
	J8	0.1753	—	1	—	—	—	—	—	98.6
	J9	0.1753	—	1	—	—	—	—	—	98.4
Associate Referee	K1	0.1765	—	1	—	—	—	—	—	102.8
	K2	0.1765	—	1	—	—	—	—	—	102.9
I. S. Shupe	K3	0.1765	5	—	18.35	0.40	0.00	20	20	98.3
	L1	0.1752	5	—	18.59	0.53	0.00	20	20	101.0
Associate Referee	L2	0.1752	—	1	—	—	—	—	—	100.6
	L3	0.1752	—	1	—	—	—	—	—	100.5
I. S. Shupe	L4	0.1752	—	1	—	—	—	—	—	98.3
	L5	0.1752	—	1	—	—	—	—	—	98.2

* J1, J2, etc., each represents a 5 cc. aliquot of the same 100 cc. ether-water solution, which contained the given quantity of ether per 5 cc. of solution, when prepared by the associate referee.

† Quantity of alcohol in cylinder B to which the 5 cc. aliquot of ether-water solution was added.

certain all connections are air-tight. (Usually stopcock L requires no further adjustment.) Carefully adjust stopcock K until a rapid and steady current of bubbles (about 150 per minute) flows through the aspiration train. (Usually this is attained with cock K slightly open, depending upon the size of the opening through cock L.) Take care not to have any of Reagent (c) touch the rubber stopper by spray or otherwise. As they rise in cylinders B and C the bubbles increase in size, couple up, and near the surface each bubble occupies the entire cross-section of the cylinder and has a vertical height of 1–1.5 cm.)

Aspirate for 5 hours. If not certain that all the ether has been carried over into the 0.5 *N* acid-dichromate solution, discontinue the aspiration as directed in next paragraph. Transfer the contents of cylinder *E* into a ground-glass stoppered 500 cc. volumetric flask. Pipet 25 cc. of Reagent (c) into cylinder E. Aspirate as before.

Gradually open cock K until the rate of flow of the bubbles is appreciably slower and disconnect the rubber tubing between cylinders B and C. Then gradually open cock K as before and disconnect the tubing between C and D. Repeat until all cylinders are disconnected.

Transfer the acid-dichromate solution (contents of cylinders E, F and G) into a ground-glass stoppered 500 cc. volumetric flask. Wash the cylinders and glass tubes with water and drain washings into the flask. Add 200–300 cc. of water and cool. Add more water and again cool to room temperature. Make up to volume and mix thoroughly.

Pipet a 25 cc. aliquot into a 500 cc. ground-glass stoppered flask and continue as directed under Reagent (c), beginning with "add 100 cc. of water —."

Calculate the 0.5 *N* sulfuric acid-potassium dichromate solution consumed by the sample. One cc. of 0.5 *N* acid-dichromate = 4.628 mg. of ether.

COMMENTS OF COLLABORATORS

J. Carol.—The procedure is quite satisfactory. It is easily operated and requires little or no attention during the period of aspiration.

Henry R. Bond.—I have no adverse comments to make.

I. S. Shupe.—It is necessary to follow directions closely throughout the procedure.

GENERAL REMARKS

The results obtained, which were above 100 per cent, may be accounted for either by the possibility that the aliquot of the aqueous ether solution, drawn in a pipet, contains a higher percentage of ether than the sample as a whole, or, in practice the oxidation of the ether at times goes slightly beyond acetic acid or partially to carbon dioxide and water. The first possibility appears somewhat plausible because the first 2 or 3 aliquots taken from a sample very consistently yield over 100 per cent recovery. Each successive aliquot from the same sample gives successively lower percentages of ether, which may be expected because of a loss of ether by volatilization. The second possibility (oxidation of the ether partially beyond acetic acid) is somewhat confirmed by four out of five experiments, in which the absolute ether samples weighed in a small weighing flask were transferred into a flask containing 50 cc. of 0.5 *N* acid-dichromate, Reagent (c), and left to stand at room temperature for 1 to 16 hours. The results obtained were 99.3, 105.2, 106.2, 111.9 and 112.2

per cent recovery. The conditions of these experiments are not the same as those of the proposed method.

Perhaps the period of aspiration might be shortened by mixing the ether-alcohol-water sample with sulfuric acid (1+1) in cylinder B and eliminating one of the two cylinders with its alcohol absorption solution (sulfuric acid, 1+1).

The results obtained by the separation and determination of ether in ether-alcohol-water solutions (using aspiration) were as good as the results obtained by the determination of ether in aqueous solutions directly in a flask, without aspiration. This suggests that details other than the separation of the ether from the alcohol, by absorption of the latter, possibly the exact temperature range within which the oxidation of the ether does not proceed beyond acetic acid, require more study if greater accuracy is required.

SUMMARY

A simple, practical, and reasonably accurage method for the determination of ether was devised by the associate referee. It is applicable to ether and to aqueous, alcoholic or hydro-alcoholic solutions of ether containing, within very wide limits, varying quantities of ether, alcohol and water.

The procedure is based upon the quantitative oxidation of ether by 0.5 N acid-dichromate, Reagent (c), under certain conditions, and the separation of ether from alcohol, aspiration with air being used to carry the ether vapor through sulfuric acid (1+1), which absorbs the accompanying alcohol vapor, into the oxidation reagent.

The directions given, without exception, should be carefully followed. Ether is very volatile and accordingly it or its solution must be handled carefully. Appreciable deviations from the details of the method, particularly in the acidity of the acid-dichromate reagent and in the length of the time of aspiration, will give erroneous results.

It may be remarked that while the proposed method is inherently time-consuming, the actual working time required (other than the period of aspiration) is about 1 hour.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method developed by the associate referee for the determination of ether, which is applicable to ether and aqueous, alcoholic or hydro-alcoholic solutions of ether, be adopted as a tentative method.

(2) That the quantitative determination of ether in mixtures other than water and alcohol be studied.

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[1] For report of Subcommittee B and action of the Association, see *This Journal*, 16, 52 (1933).

REPORT ON BENZYL COMPOUNDS

By Joseph Callaway, Jr.[1] (U. S. Food and Drug Administration, New York, N. Y.), *Associate Referee*

This Association undertook the study of methods of analysis of benzyl compounds for the first time this year. An investigation of products that might be classified under this heading indicated that certain esters of benzyl alcohol are more extensively used as drugs than are the other ordinary benzyl compounds. These esters are usually dispensed in solution in an oil. Benzyl alcohol itself is probably the most important of the benzyl compounds other than esters as a therapeutic agent. It is usually dispensed in dilute aqueous solution or in physiological salt solution.

In collaboration with Solomon Reznek of this Station,[2] a study was made of some of the physical and chemical properties of benzyl aclohol and a method for its determination was outlined. In order to test this method three samples were prepared for collaborative study by dissolving benzyl alcohol in water and salt solution as follows:

1. Solution of benzyl alcohol in water containing 1.87 grams per 100 cc. of benzyl alcohol.

2. Solution of benzyl alcohol in aqueous salt solution (1%) containing 0.96 gram per 100 cc. of benzyl alcohol.

3. Solution of benzyl alcohol in water containing 3.47 grams per 100 cc. of benzyl alcohol.

These samples were sent to collaborators with requests that benzyl alcohol be determined by the method presented by Callaway and Reznek.[3]

The results on the following page were obtained by the collaborators.

It will be seen that the results obtained from refractometer readings agree closely with the theoretical. Results secured by oxidizing to benzoic acid show a recovery of about 95 per cent. It is believed that the method will be found satisfactory if benzyl alcohol is calculated from the refractometer reading and this checked by the oxidation to benzoic acid, this latter figure being multiplied by 1.05 to compensate for loss on oxidation.

Since, however, the more important benzyl compounds, insofar as drug use is concerned, are probably the benzyl esters, particularly benzyl benzoate, it is recommended[4] that next year a study be made of a suitable method for determining benzyl benzoate, and that, if possible, the method cited for the determination of the alcohol radical of this ester be used.

No report on alcohol in drugs was given by the associate referee.

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[1] Presented by J. F. Clevenger.
[2] *This Journal*, 16, 285 (1933).
[3] *Loc. cit.*
[4] For report of Subcommittee B and action of the Association, see *This Journal*, 16, 52 (1933).

COLLABORATORS	SAMPLE A 1.872 GRAMS PER 100 CC.				SAMPLE B 0.965 GRAM PER 100 CC. plus 1% SALT				SAMPLE C 3.474 GRAMS PER 100 CC.			
	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)	(1)	(2)	(3)	(4)
Bruening, F. & D. Adm. Baltimore	1.852	2.142	1.793	95.8	1.004	1.342	0.944	97.8	3.474	3.514	3.327	95.8
			1.785	95.4			0.939	97.3			3.362	96.8
Eaton, F. & D. Adm. San Francisco	1.85		1.77	94.6	1.00		0.91	94.3	3.47		3.30	95.0
Reznek, F. & D. Adm. New York	1.856	1.62	1.778	95.0	0.965	0.7	0.926	96.3	3.474	3.2	3.310	95.3
			1.778	95.0			0.929	96.3			3.328	95.8
Cannon, F. & D. Adm. Chicago	1.79	2−	1.76	94.0	0.960	0.6	0.931	96.5	3.27	3.	3.38	97.3
	1.78	1+	1.78	95.1	0.960	0.7	0.905	93.8	3.28	3.	3.26	93.8
	1.79	2−			0.958	0.8			3.27	3.	3.24	93.3

(1) Grams per 100 cc. benzyl alcohol calculated from immersion refractometer reading.
(2) Grams per 100 cc. benzyl alcohol calculated from specific gravity.
(3) Grams per 100 cc. benzyl alcohol calculated from benzoic acid obtained by oxidation.
(4) Per cent recovery by oxidation to benzoic acid.

REPORT ON SMALL QUANTITIES OF IODIDES IN MIXTURES

By FRANK C. SINTON (U. S. Food and Drug Administration, New York), *Associate Referee*

Last year a collaborative study was made and a method was adopted for the determination of substantial quantities of iodine in mixtures. It was recommended by Subcommittee B that the topic be continued this year with special reference to the determination of minute proportions of iodine.

A study of the literature revealed that H. A. Halvorson, Associate Referee on Mineral Mixed Feeds, is working on methods for the determination of small quantities of iodides in mixtures. As it was considered that his work would yield satisfactory methods no further work was done by the associate referee. It is recommended[1] that the topic be dropped.

No report was given on bismuth compounds in tablets as the subject was closed last year.

REPORT ON PHENOLSULFONATES

By ERNEST H. GRANT (U. S. Food and Drug Administration, Baltimore, Md.), *Associate Referee*

As recommended last year, the method then developed and since published[2] was submitted to collaborators, together with a sample of commercial sodium phenolsulfonate and another sample consisting of 46 per cent of this salt and 54 per cent of lactose.

Results were received from ten collaborators:

Collaborative results.

COLLABORATOR	USING 0.4N Br		USING 0.1N Br	
	SAMPLE A	SAMPLE B	SAMPLE A	SAMPLE B
	per cent	*per cent*	*per cent*	*per cent*
George E. Éwe	98.0	45.9	99.2	45.6
Tailby Nason Co.	99.1	45.9	99.2	45.7
Boston	98.0	45.6		
	98.6			
Harry J. Fisher	100.4	46.78	101.7	46.23
Conn. Agr. Expt. Sta.	100.4	46.63	101.5	46.53
New Haven				
Samuel H. Culter	100.03	46.7		
Burroughs Wellcome Co.	100.12	46.4		
Tuckahoe, N. Y.				

[1] For report of Subcommittee B and action of the Association, see *This Journal*, 16, 53 (1933).
[2] *This Journal*, 16, 83 (1933).

COLLABORATOR	USING 0.4N Br		USING 0.1N Br	
	SAMPLE A	SAMPLE B	SAMPLE A	SAMPLE B
	per cent	*per cent*	*per cent*	*per cent*
F. E. Willson	99.82	46.55		
Parke, Davis & Co.	99.93	46.67		
Detroit		46.67		
John C. Krantz, Jr.	100.0	45.9		
Maryland Dept. Health	99.8	46.1		
Baltimore				
L. D. Seif	99.79	45.99	99.19	45.91
Wm. S. Merrell Co.	99.36	45.99	99.05	45.98
Cincinnati, Ohio				
S. M. Berman	99.42	45.78	97.87	45.11
U. S. Food & Drug Adm.	99.17	45.99	98.34	45.43
New York	99.44	45.86	98.53	46.05
	99.45		98.65	46.02
	99.40			
James Boyd	99.94	46.11		
Mallinckrodt Chem. Works	99.95	46.14		
St. Louis	99.96			
Albert P. Blaisdell	98.96	45.33		
Bur. Int. Revenue	99.24	45.19		
Washington	99.26	45.64		
Abraham Van Loo	99.87	45.72		
Mich. Dept. Agr.	99.61	45.90		
Lansing				

As mentioned in previous reports, the end point is a little difficult to recognize. Several collaborators reported trouble in this respect. However, with experience, an analyst can obtain reasonably accurate results, that is, within about 1 per cent either way. Undoubtedly the chemists that submitted low results would have obtained satisfactory ones had they made sufficient titrations to become familiar with the end point.

The associate referee is submitting this method with the comment, therefore, that an analyst should thoroughly familiarize himself with the end point before attempting to use the method regularly. The method is not applicable in the presence of other substances that brominate rapidly. It is not invalidated by substances which brominate very slowly, nor by the presence of many catalysts, as is the former U. S. P. method.[1]

The same bonds that are attacked by bromine also react with oxidizing and other reagents. Several oxidizing reagents were tried on phenolsulfonates, but all seemed to act in the same indefinite manner as bromine, and no conditions were discovered which would give definite, satisfactory results.

[1] United States Pharmacopoeia, IX, p. 395.

Thanks are extended to the collaborators, to Joseph Rosin of Merck & Co., Rahway, N. J., who volunterred to furnish supplies of the phenol-sulfonates of zinc, sodium, and calcium specially purified for this investigation, and to the chemists of the same company who purified the salts.

It is recommended that this method be adopted as tentative.

REPORT ON SULFONAL (SULFONMETHANE) AND TRIONAL (SULFONETHYLMETHANE)

By W. S. HUBBARD, *Associate Referee*, and C. A. DAYHARSH (Schwarz Laboratories, Inc., New York, N. Y.)

Last year's work on the methods for determination of sulfonal and trional reported by the associate referee seemed more consistent than did the previous year's work, and it was recommended "that the methods described be studied collaboratively with the use of ethyl ether and drying the residue 18 hours in a desiccator," and "that the melting point determination of the trional recovered be made."

The methods submitted to the collaborators were practically the same as those submitted last year. The results are shown in the accompanying tables.

Trional and Sulfonal

ANALYST	SAMPLE AND METHOD	RECOVERED	PERCENTAGE RECOVERY	UNCORRECTED MELTING POINT OF RECOVERED MATERIAL
		per cent		*°C.*
	Trional Method I.—			
H. J. Fisher	Mixture	49.08	102.43	75
Conn. Exp. Station	I (M.P. 76°C):	49.28	102.85	73
New Haven	Trional, 19.8762 grams			
W. F. Reindollar	Starch, 21.6050 grams	48.60	101.43	73.1
Dept. of Health		48.65	101.53	73.1
Baltimore		48.35	100.91	
A. Van Loo		48.62	101.47	74
Dept. of Agr.				
Lansing, Mich.				
C. A. Dayharsh		48.54	101.30	75.5
Schwarz Labs.		48.15	101.49	75.0
New York City		47.75	101.74	73–74
L. E. Warren		47.96	102.18	
U. S. Dept. Agr.		48.44	101.09	
Washington				
	Average	48.67	101.58	

ANALYST	SAMPLE AND METHOD	RECOVERED	PERCENTAGE RECOVERY	UNCORRECTED MELTING POINT OF RECOVERED MATERIAL
		per cent		*C.*
Fisher	Method II	47.84	99.84	75.0
	Same mixture	47.72	99.59	77.0
Reindollar		50.07	104.50	73.1
		48.46	101.14	73.2
		49.14	102.55	
Van Loo		49.14	102.55	74.0
Dayharsh		48.31	100.82	75.75
Warren		48.25	100.70	73–74
		48.01	100.20	
	Average	48.55	101.32	
C. S. Leonard	Method I	62.20	102.43	
Burroughs Wellcome	Trional Mixture II	62.21	102.44	
Lab.	Trional, 18.8108 grams			
Tuckahoe, N. Y.	Starch, 12.1647 grams			
A. A. Jackson		62.60	103.08	75.5
Schwarz Labs.				
New York City				
H. P. Strack		61.91	101.95	
Dept. of Agr.		61.72	101.64	
Nashville				
Strack		61.75	101.68	
		62.26	102.52	
		61.93	101.98	
	Average	62.07	102.22	
Leonard	Method II	61.67	101.55	
	Same mixture	61.48	101.24	
		61.58	101.40	
		61.67	101.55	
Jackson		62.24	102.49	75.5
Dayharsh		61.19	100.76	
	Average	61.64	101.50	

ANALYST	SAMPLE AND METHOD	RECOVERED	PERCENTAGE RECOVERY	UNCORRECTED MELTING POINT OF RECOVERED MATERIAL
		per cent		*°C.*
Fisher	Method I	56.34	103.18	125.5
	Sulfonal Mixture:	55.94	102.45	125.0
Reindollar	Sulfonal, 24.4810 grams	55.22	101.13	124.8
	Starch, 20.3524 grams	55.31	101.29	124.6
		55.12	100.94	
Leonard		56.72	103.88	
		56.20	102.92	
Jackson		55.82	102.23	126
Van Loo		57.54	105.38	126.5
Dayharsh		55.81	102.21	126.25
Warren		55.09	100.90	125–125.5
		55.22	101.22	
	Average	55.87	102.31	
Fisher	Method II	55.48	101.60	125.5
	Same Mixture	55.26	101.20	126.5
Reindollar		55.76	102.12	125.0
		55.68	101.97	125.0
		56.29	103.09	
Leonard		55.93	102.43	
		56.09	102.72	
Jackson		55.69	101.99	
Van Loo		57.36	105.05	126
Dayharsh		54.57	99.94	126.0
		53.66	98.28	126.25
Warren		55.77	102.14	125–125.5
		55.60	101.82	
	Average	55.55	101.87	

COMMENTS OF COLLABORATORS

Wm. F. Reindollar.—(Trional): Method I, in my hands, yields more consistent results. I believe the residue should be dried to constant weight rather than for an arbitrary period, as further drying over concentrated sulfuric acid causes a slight decrease in weight. (Sulfonal): In Method II, I found it necessary to rinse the tip

of the Knorr tube with ether as evaporation of the solvent caused a deposit to form there. Sulfonal appears to be much more soluble in warm than in cold ether, and this condition, I believe, favors Method I.

DISCUSSION

The results of the collaborators are consistently high, and considerable thought has been given to this fact. The trional and sulfonal conformed to the standards of the U. S. Pharmacopoeia, with the melting points at the upper limit given and the melting points of the collaborators checked this figure quite closely. One of the authors (C. A. Dayharsh), made an examination of the extracted material to be sure nothing was coming from the starch. It could hardly come from the sand for the different collaborators used different sand, and in the laboratory of the associate referee different qualities of sand had been tried with the same results. Absolute ether gave no better results than the ordinary U.S.P. ether.

A. A. Jackson has pointed out that it is a quite common practice to first put the extraction flask in an oven at 100° C., cool in a desiccator, and then weigh and take this weight as the tare of the flasks. He has shown that these flasks will take on weight upon standing in the desiccator. It would seem, therefore, that the tare of the flasks should be obtained under the same conditions as exist when the trional and sulfonal are weighed. Also, it would appear from the work of the year 1931 that drying the trional or sulfonal at 37° C. and then in a desiccator would give the best results.

There was not time after the reports of the collaborators were received to do any further work along this line.

As stated last year, it is realized that trional and sulfonal are of little commercial importance at the present time, due to the use of more important drugs.

Therefore, it is recommended:[1]

(1) That the methods submitted for the determination of trional and sulfonal be published but not adopted.

(2) That no further work be done on trional and sulfonal at this time.

REPORT ON GUAIACOL

By N. L. KNIGHT (U. S. Food and Drug Administration, St. Louis, Mo.),
Associate Referee

In the previous report on guaiacol,[2] attention was directed to the fact that this compound may be dissolved in water by boiling it under a reflux condenser, and that there is no subsequent separation on cooling.

[1] For report of Subcommittee B and action of the Association, see *This Journal*, 16, 53, 83 (1933).
[2] *This Journal*, 15, 429 (1932).

Guaiacol solutions prepared in this manner were used for most of the work discussed in the present report, except that in a few cases an alkaline aqueous solution several months old was used for purposes of comparison. The method of preparing the alkaline solution was described last year.

An experiment described later demonstrates that the brown discoloration developed by alkaline aqueous guaiacol solutions on standing is caused by their alkalinity; possibly it is the result of oxidation. Since this extraneous reaction might interfere with the determination of guaiacol by methods previously tried, some of the previous work was repeated, the new type aqueous solution prepared by boiling being used. The guaiacol itself had recently been re-distilled and was colorless.

These additional tests with the new solution were applied to the bromate-thiosulfate method; to the bromate titration method, the bleaching of an indicator being used as the end-point; and to the color-comparison method. A method heretofore untried by the associate referee was also investigated (acetylation in pyridine solution under pressure), and several miscellaneous experiments were performed.

I. *Bromate-Thiosulfate Method.*—Some preliminary work on this method was described in the initial report on guaiacol.[1] Aliquots of the aqueous guaiacol solution were treated with measured quantities of concentrated hydrochloric acid, distilled water heated to 85° C., and an excess of 0.1N sodium bromate solution. In most of the trials the solution was warmed during a variable interval on the steam bath, then 5 cc. of potassium iodide solution (equivalent to 1 gram of KI) was added, together with a few cc. of starch solution, and the mixture was titrated with standard sodium thiosulfate solution until the blue color disappeared.

The vessel used for the bromination in some cases was an unstoppered Erlenmeyer flask; in others, a pressure flask with the type of stopper used on magnesium citrate bottles, fitted with a rubber gasket; and in a few others, a wide-mouthed, glass-stoppered bottle tightly packed in cotton and enclosed in an aluminum cylinder separable into halves by unscrewing, such as is used for sterilizing bacteriological sample bottles. The last-mentioned type of vessel was used, as it was thought that the bromine might be reacting with the rubber gasket of the pressure flask.

An alkaline solution of guaiacol, about eight months old and deep brown in color, was also treated by this method. The results show wide variation, and a tendency to greater bromination with prolonged heating is evident. The old alkaline solution shows greater variation than the other.

It appears that bromination in warm solution, although applicable to thymol,[2] is not successful in the case of guaiacol. Buckwalter and Wagner[3]

[1] *This Journal*, 14, 367 (1931).
[2] Hart, F. L., *This Journal*, 12, 55 (1929).
[3] *J. Am. Chem. Soc.*, 52, 5241 (1930).

state that some substances brominate normally at room-temperature, others at 75° C., while others yield entirely meaningless results unless brominated near 0° C., which for some substances is perhaps still too

TABLE 1

Guaiacol by bromate titration.

(Bleaching of various indicators as end point.)

INDICATOR USED	ALIQUOT TAKEN	GUAIACOL PRESENT	GUAIACOL DETERMINED	ERROR	REMARKS
	cc.	gram	gram	per cent	
Methyl Green	50	0.00845	0.00925	+9.5	Initial color very pale; indefinite end point
Methyl Violet	50	0.00845	0.00843	−0.2	Most definite end point of any of the indicators tried
Methyl Violet	50	0.00845	0.00859	+1.7	—
Methyl Violet	50	0.00845	0.00859	+1.7	—
Benzo-Purpurin	50	0.00845	0.00859	+1.7	End point next to methyl violet in sharpness
Benzo-Purpurin	100	0.0169	0.01669	−1.2	—
Methyl Orange	50	0.00845	0.00909	+7.6	Indefinite end point; obscured by the usual chocolate-brown color
Fuchsine	100	0.0169	—	—	Color did not bleach even with excess bromate; titration at room temperature
Fuchsine	100	0.0169	—	—	Hot H_2O used; color vanished before bromate was added
None	50	0.00845	—	—	No end point; only slow intensification of chocolate-brown color

high a temperature. It is planned to follow up this observation by repeating the work, but carrying out the bromination at or near 0° C.

II. *Sodium Bromate Titration.*—This is also a modification of the method

for thymol. Methyl orange and benzo-purpurin having been found unsatisfactory as indicators owing to the masking of the end point by the chocolate-brown color developed during the bromination, trials of other indicators were made. The results are given in Table 1.

Since methyl violet gave a color whose disappearance in the presence of the brown bromination product could be more easily detected than any of the others, it was used in a series of determinations. Wide variation was noted here also. After a series of titrations with the mixture at an initial temperature of approximately 85° C., another series was made. Water at 15° C. was used and a cube of ice was added. Even greater variation was obtained by the latter method.

Francis[1] states that "pyrocatechol" (synonymous with catechol and pyrocatechin) is oxidized and the molecule destroyed by bromination at room temperatures, giving a dark color which obscures the end point, but that this may be avoided by titrating below 0°C., with ice in the solution. Day and Taggart[2] also note the great readiness of certain aromatic compounds to be oxidized by aqueous bromine, and recommend cooling to 0° C., or below as a measure which sometimes prevents this reaction. They state that one difficulty of any bromination method is the appearance of reactions other than those expected. Additional work on this method is also planned, with bromination performed at or near 0° C.

III. *Color Comparison Method.*—Previous experience had caused this method to be placed in temporary abeyance. However, the work that was done seems to indicate that the new guaiacol solution gives more uniform results.

Resorcinol and hydroquinone were used as standards, and single trials were made of two Eastman photographic reagents, "Elon" and "Photol," the formulas of which are at present unknown. However, they gave color intensities with the Denis-Folin reagent[3] nearer that given by guaiacol than did resorcinol or hydroquinone, but shared with the latter substance the disadvantage of rapidly aquiring a tinge of color on standing 24 hours—making necessary the daily preparation of fresh standard solutions during continued work. Additional work will be done on this method; the scant and fragmentary data now at hand do not warrant tabulation.

IV. *Acetylation Under Pressure.*—This procedure was used by Peterson and West[4] and by Marks and Morrell[4] for the determination of hydroxyl in organic compounds. The substance is treated with a mixture of one volume of acetic anhydride and two volumes of pyridine, the latter absorbing the acid formed.

The reaction product is poured into ice water and titrated with alkali, phenolphthalein being used as indicator. A blank consisting of the acetic

[1] *J. Am. Chem. Soc.*, 46, 2498 (1924).
[2] *Ibid.*, 20, 545 (1928).
[3] *This Journal*, 15, 430 (1932).
[4] *J. Biol. Chem.*, 74, 379 (1927).
[5] *Analyst*, 56, 428 (1931).

anhydride-pyridine mixture is subjected to the same treatment. The difference between the titration of sample and blank represents the amount of acetic anhydride consumed in acetylating the compound.

The vessels used for the acetylation were the same wide-mouthed glass-stoppered bottles, enclosed in aluminum cylinders, as were used for bromination under pressure. The stoppers were first tightly ground in with emery flour to give a perfect fit and were tied on while the bottles were in use. As an additional preventive of blowing out the stoppers, the bottles were padded with cotton at both ends so that when the two halves of the aluminum cylinder were screwed together the bottles were held shut by the compression of the cotton. The entire assembly was then lowered into the steam bath on a string and removed in the same manner.

The results obtained with guaiacol are given in Table 2. Peterson and West did not apply the method to guaiacol, but determined the hydroxyl

TABLE 2

Guaiacol by pressure-flask acetylation.
(Inside temperature of steam bath, 97°C.)

TIME		HOW COOLED	GUAIACOL PRESENT	GUAIACOL DETERMINED	ERROR	
AT ROOM TEMP.	IN STEAM BATH					
			gram	*gram*	*gram*	*per cent*
None	72 min.	Cooling trough	0.3165	0.3198	+0.0033	+1.0
None	72 min.	Cooling trough	0.1805	0.1874	+0.0069	+3.8
None	30 min.	Cooling trough	0.0335	0.0331	−0.0004	−1.3
None	45 min.	Room-Temp. 3¾ hrs.	0.0375	0.0414	+0.0039	+10.3
None	45 min.	Room-Temp. 3¾ hrs.	0.0335	0.0276	−0.0059	−17.7
3 hrs.	45 min.	Room-Temp. 30 min.	0.0355	0.0331	−0.0024	−6.8
3 hrs.	45 min.	Room-Temp. 30 min.	0.0365	0.0331	−0.0034	−9.4
None	1¾ hr.	Cooling trough	0.0350	0.0303	−0.0047	−13.4
					Average Error:	±7.4

in allied compounds, such as resorcinol and hydroquinone, using samples ranging from 0.1 to 0.8 gram. Their tabulation shows errors varying from +2.2 to −4.0 per cent, with an average of ±1.7 per cent and the smallest +0.3 per cent. It will be noted that the first two samples of guaiacol in Table 2 (approximately 0.3 and 0.2 gram., and within the weight-range of Peterson and West's samples) give comparable errors, but that the smaller samples give much wider deviations.

Marks and Morrell included guaiacol in their series of substances upon which hydroxyl was determined, and their smallest error was −0.2 per

cent, or −1.46 per cent relative to the quantity being determined, i.e., the 13.7 per cent of hydroxyl. These two workers used samples ranging in weight from 0.5 to 1.0 gram.

MISCELLANEOUS EXPERIMENTS

1. *Determination of Guaiacol Carbonate by Bromination.*—Reference has been made in previous reports to the method of Chernoff[1] for determining guaiacol carbonate by precipitation as the crystalline monobrom compound, and to the failure of the associate referee to obtain such a precipitate when the method was applied to guaiacol. In order to show that this failure was not due to some error in handling the procedure, the associate referee analyzed four samples of guaiacol carbonate. The results were satisfactory. The crystalline precipitate was readily formed and easily filtered.

2. *Qualitative Tests with Chloramine-T.*—This experiment was suggested by a certain test for resorcinol.[2] About 1 cc. of a cold saturated solution of chloramine-T was added to 4 cc. of approximately 10 per cent solutions of the compounds tested. Guaiacol did not give a crystalline precipitate.

3. *Discoloration of Alkaline Guaiacol Solutions.*—A reference in the literature to the bluish-green discoloration of a prescription containing tincture of guaiacol and sirup of acacia by Krantz and Carr,[3] which was traced to the action of an enzyme introduced by the latter constituent, suggested that the brown discoloration of dilute alkaline guaiacol solutions might be due to a similar ferment deposited from the air. To clear up this point the following experiment was made:

Three small flasks were sterilized with boiling distilled water. In one a small quantity of guaiacol was shaken vigorously with about 25 cc. of boiled and cooled distilled water and allowed to stand in the unstoppered flask. The temporary emulsion soon broke and most of the guaiacol separated in globules on the bottom of the flask. An aqueous solution of guaiacol was prepared by boiling under a reflux condenser, and small quantitites were introduced into each of the other two sterilized flasks, one of which was allowed to stand unstoppered, and the other was plugged with sterile absorbent cotton. At the end of 38 days the contents of all three flasks were still colorless.

An approximately 10 per cent solution of sodium hydroxide was then heated to boiling, and a portion was filtered while hot into each of the three flasks. Within one hour after adding the sodium hydroxide a brown coloration had appeared in all three flasks. It appears that the discoloration is due to purely chemical action—probably oxidation—taking place

[1] *J. Am. Chem. Soc.*, **51**, 3072 (1929).
[2] Allen. Commercial Organic Analysis, 5th ed. vol. III, p. 336; Berthelot and Michel. Bull. sci. pharmacol., **26**, 401 (1919).
[3] *J. Am. Pharm. Assoc.*, **18**, 1153 (1929).

in alkaline but not neutral solutions, and is not due to the action of an enzyme initially present in the guaiacol or introduced from the air.

SUGGESTIONS FOR FUTURE WORK

It is planned to do some additional work (1) on the bromate titration method (using methyl violet as the indicator) and the bromate-thiosulfate method, performing the bromination at temperatures around 0°C.; (2) on the color-comparison method, using the aqueous solution of guaiacol prepared by boiling. Two different lines of investigation may be followed:

(a) The method previously tried, using the Denis-Folin reagent and standard solutions of resorcinol and hydroquinone—or other substances which may prove suitable.

(b) The method of Schmalfluss, Spitzer, and Brandes,[1] utilizing the formation of a colored compounds of guaiacol with ferric iron, with guaiacol itself as the standard.

The precipitation of guaiacol carbonate with formaldehyde has been reported by Ware,[2] and the method may be applicable to guaiacol, although the abstract of the paper makes no mention of it.

Crystalline precipitates have been obtained from the reaction between pyrocatechol acetate in saturated solution in pure aniline, and ammonium molybdate or sodium tungstate, according to the findings of Martini.[3] The abstract does not state whether or not the precipitation is quantitative, nor whether tests were made on guaiacol.

The direct titration of guaiacol with sodium or potassium hydroxide is also a possible method. Christie and Menzies[4] report that the titration of guaiacol and other analogous compounds with thallous hydroxide (TlOH) "is as accurate as the titration with NaOH or KOH, the equivalents of the acids determined being correct to three significant figures."

It is also planned to make a few tests of these three methods.

It is recommended[5] that this study be continued.

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No report on bromide-bromate methods was given by the associate referee.

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REPORT ON IPOMEA, JALAP, AND PODOPHYLLUM

By L. E. WARREN (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

An associate referee on these subjects was appointed at the 1930 meeting.[6] Because of the chemical similarity of ipomea and jalap these two

[1] *Biochem. Z.*, **189**, 226 (1927).
[2] *Quart. J. Pharmacol.*, **2**, 249, 254, 265 (1929).
[3] *Anales asoc. quim. Argentina*, **14**, 177 (1926); *Mikrochemie*, **6**, 63 (1928); *Chem. Zentr.*, 1928, 1 (1894).
[4] *J. Chem. Soc.*, **127**, 2369 (1925).
[5] For report of Subcommittee B and action of the Association, see *This Journal*, **16**, 53 (1933).
[6] *This Journal*, **14**, 7 (1931).

drugs were considered together as a sub-topic, and podophyllum was treated separately.

IPOMEA AND JALAP

Before the associate referee was appointed two samples of ipomea and three of jalap had been assayed by several collaborators by a method which had previously been found by the writer to be satisfactory.[1] This method is an adaptation of the U.S.P.X. process for extracting the drug and the method of the French Pharmacopoeia for purifying the resin. However, the results obtained were not reported at the 1930 meeting because no comparison had been made in a collaborative way between the U.S.P.X. method and the proposed process. Last year the work on ipomea and jalap[2] consisted in making collaborative trials of these two methods of assay. The results indicated that the U.S.P.X. method was unsatisfactory. The other process gave consistent results.

While the work was in progress last year, however, the associate referee was informed by W. L. Scoville[3] that alcohol of lower strength than 94.9 by volume would extract more resin from jalap than would the solvent of U.S.P.X. standard. Scoville believed that about 75 per cent alcohol was the optimum strength to use. As the time last year did not permit of a study of this phase of the subject, the topic was continued.[4]

TABLE 1
Assays of ipomea, percentage of resin.

ALCOHOL (per cent)	SAMPLE D				SAMPLE E			
	94.9	85.4	76.5	68.8	94.6	85.4	76.5	68.8
COLLABORATOR								
A					14.48	14.25	14.11	14.02
					14.59	14.17	14.13	14.10
B	16.20	16.31	16.40	15.90	14.41	14.54	14.40	13.60
	16.31	16.28	16.35	15.60	14.24	14.56	14.59	13.50
	16.78	16.46						
C	16.77	16.43	16.57	15.29	14.54	14.16	14.07	13.82
	16.87	16.32	16.48	15.42	14.50	14.42	13.96	14.06
	16.97	16.32	16.50			14.36	14.69	
						14.27	14.69	
							14.70	

This year specimens of ipomea and jalap were assayed by the modified U.S.P.X. method for extraction and the method of the French Pharmacopeia for purification of the resin. Alcohol of approximately 95, 85, 75, 68 and 50 per cent strengths was used as solvent. Preliminary studies

[1] *This Journal*, 12, 324 (1929); 13, 377 (1930).
[2] *Ibid.*, 15, 448 (1932).
[3] Personal communication.
[4] *This Journal*, 15, 452 (1932).

demonstrated that 50 per cent alcohol was impractical, consequently the collaborators were not requested to use this strength. In some instances the extracts were assayed for total solids by evaporation and drying the residue at 80°. Within the limits of the strengths of alcohol used the results indicated that, in general, the total solids increase inversely as the alcoholic strength of solvent employed. The findings for total solids extracted are not published as they have but little bearing on the assay for resins. The extracts were assayed for resin by the washing method found satisfactory last year.[1] The results obtained for resin are given in Tables 1 and 2.

TABLE 2
Assays of Jalap, percentage of resin.

ALCOHOL (per cent)	SAMPLE D				SAMPLE E			
	94.9	85 4	76.5	68.8	94.9	85.4	76.5	68.8
COLLABORATOR								
A					16.64	16.55	16.38	16.23
					16.76	16.50	16.34	16.21
B	12.21	12.14	11.72	11.73	16.40	16.11	16.31	15.35
	12.24	12.03	12.26	11.92	16.19	16.90	16.32	15.82
			12.24				16.28	15.34
							16.27	
C	12.56	12.30	12.23	11.76	16.51	16.48	15.91	16.10
	12.42	12.35	12.14	11.94	16.43	16.42	15.88	16.32
				12.11		16.38		16.20
						16.51		

COMMENTS OF COLLABORATORS

A.—The lesser strengths of alcohol appear to extract the resins (from ipomea and jalap) satisfactorily, although the yield is slightly lower. However, a quantity of nonresinous matter is also obtained with these solvents. This is especially noticeable in the case of ipomea.

B.—Alcohol of lower strengths down to about 75 per cent apparently extracts the resin from ipomea and jalap nearly as completely as alcohol of 95 per cent. Below 70 per cent the resin is not completely extracted. However, with the lower strength alcohols much more extractive matter other than resin is removed. Further, these extractives are much more difficult to purify by the washing process than those obtained with 95 per cent alcohol. The extractive with 50 per cent alcohol is so difficult to purify that the use of that solvent in the assay of these drugs appears to be unjustifiable.

Preliminary trials by the associate referee demonstrated that alcohol of 75 per cent strength extracts about as much resin from both ipomea and jalap as does the 95 per cent strength; 68 per cent alcohol does not extract as much resin as alcohol of higher strengths, and 50 per cent ex-

[1] *This Journal*, 15, 448 (1932).

tracts still less. However, the residues from the extraction with the lower strength alcohols contain so much more extractives other than resin that they are not readily purified with hot water. Consequently, the use of alcohol of lower strength than 94.9 per cent is not recommended.

The method found satisfactory for the assay of ipomea and jalap has been published.[1]

It is recommended[2] that the method submitted for the assay of ipomea and jalap be adopted as tentative.

PODOPHYLLUM

The collaborative work on podophyllum last year[3] consisted in comparing the U.S.P.X. method of assay with the Jenkins method.[4] Three specimens were assayed by four collaborators by each of the two methods. Neither method was considered satisfactory because drug dealers desire an assay method which will give results for resin comparable with those expected in manufacturing processes. Neither the U.S.P.X. process nor the Jenkins method does this, the results by each being somewhat higher than the yield obtained by manufacturers. Therefore, it was decided to continue the work in the hope of securing a method more nearly in keeping with manufacturing yields.

The method tried this year is an adaptation of the U.S.P.IX process for the production of resin of podophyllum on a commercial scale. It follows:

ASSAY

Place 10 grams of the drug in a No. 60 powder in an Erlenmeyer flask of about 250 cc. capacity and add 50 cc. of alcohol. Fit the flask with a stopper through which is inserted a glass tube about 1 meter long to act as a reflux condenser, and heat the mixture on a gently simmering steam bath for 30 minutes, shaking occasionally. Transfer the contents of the flask to a small percolator and percolate slowly with warm alcohol until about 95 cc. of tincture has been obtained. (To ascertain whether extraction is complete, collect a further 10 cc. of percolate and pour a few drops into cold water; if more than a faint cloudiness appears continue the percolation with warm alcohol until the test for resin fails. Concentrate the additional percolate by evaporation and add the residue to the flask before making up to volume). Cool the percolate to room temperature and make up the solution to 100 cc. with alcohol. Mix well.

Evaporate 50 cc. of the alcoholic solution in a beaker of suitable size until the percolate is reduced to the consistency of a thin sirup. Pour the residue slowly, with constant stirring, into a tared beaker containing 10 cc. of water previously mixed with 1 cc. of normal hydrochloric acid and cooled to a temperature below 10° C. When the precipitate has subsided, decant the supernatant liquid into a tared Gooch crucible and wash the precipitate in the beaker twice by decantation with fresh portions of 5 cc. each of cold water slightly acidulated with hydrochloric acid. Transfer the precipitate to the crucible by means of small portions of cold water slightly acidulated with hydrochloric acid. Dry the contents of the crucible at 80° C.

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[1] *This Journal*, 16, 84 (1933).
[2] For report of Subcommittee B and action of the Association, see *This Journal*, 16, 53, 84 (1933).
[3] *This Journal*, 15, 452 (1932).
[4] *Ind. Eng. Chem.*, 10, 272 (1927).

and weigh. If particles of resin adhere to the walls of the first beaker, dissolve them in warm alcohol; transfer the solution to the tared beaker, using warm alcohol for rinsing; evaporate the solution, dry the contents at 80° C.; cool; weigh; and add the total net weight to the weight of the contents of the crucible.

Two specimens of podophyllum were sent to each of four collaborators with the request that they be assayed by the method given above. Only two collaborators reported in time for their results to be recorded at this meeting. The findings did not agree very closely, consequently it was not thought worth while to give them in this report.

It is recommended[1] that the study of assay methods for podophyllum be continued.

The report on rhubarb and rhaponticum will be published later.

REPORT ON CALCIUM GLUCONATE

By HARRY J. FISHER (Agricultural Experiment Station, New Haven, Conn.), *Associate Referee*

Last year[2] a method for the determination of calcium gluconate was developed based on an estimation of the calcium content. This method, while accurate in the absence of other calcium salts, is not specific for calcium gluconate.

The observation that the optical rotation of solutions of calcium gluconate is greatly increased when the solutions are saturated with uranyl acetate led to the development of a physical method which appears to be nearly specific for calcium gluconate. The method, which has been published,[3] was submitted to collaborative study this year.

Three samples were prepared and sent to twelve collaborators, four of whom reported their results. Sample 1 was Eastman calcium gluconate which had been recrystallized from water, washed with alcohol and ether, and dried in a vacuum desiccator. Sample 2 was a mixture of one-half recrystallized calcium gluconate, one-fourth lactose and one-fourth cocoa. Sample 3 was a mixture of one-half calcium gluconate, one-fourth cocoa and one-eighth each of calcium lactate and lactose.

It was directed to analyze Sample 1 as follows:

(1) Dissolve 1 gram in water, make up to 50 cc. at 20° C. and polarize in two 200 mm. tubes. Report reading in degrees Ventzke.[4]

(2) Dissolve 1 gram in 35 cc. of water in a 50 cc. volumetric flask, add 10 grams

TABLE 1

Calcium gluconate, $Ca(C_6H_{11}O_7)_2 \cdot H_2O$.

COLLABORATORS	PERMANGANATE METHOD		OBSERVED ROTATION, 220 MM. TUBE		ROTATION PER GRAM PER 100 CC.		
	INDIVIDUAL	AVERAGE	WITHOUT URANYL ACETATE	WITH URANYL ACETATE	WITHOUT URANYL ACETATE	WITH URANYL ACETATE	DIFFERENCE
	per cent	per cent	°V.	°V.	°V	°V.	°V.
S. H. Culter Burroughs Wellcome & Co. Tuckahoe, N. Y.	86.68 86.62	86.65	+0.88	+11.53	+0.51	+6.67	+6.16
H. J. Fisher Agr. Expt. Sta. New Haven, Conn.	86.17 86.10 86.02	86.10	+1.02	+10.95	+0.59	+6.34	+5.75
M. M. Haring University of Maryland College Park, Md.	86.40 86.24	86.32	+0.95	+11.48	+0.55	+6.64	+6.09
W. F. Reindollar State Dept. of Health Baltimore, Md.	85.13 85.75	85.44	+0.90	+12.80	+0.52	+7.41	+6.89
I. S. Shupe U. S. Food and Drug Adm. Chicago, Ill.	87.44	87.44	+0.8	+11.7	+0.46	+6.77	+6.31
Average		86.39					+6.24

TABLE 2

Calcium gluconate mixtures.

COLLABORATORS	Ca(C₆H₁₁O₇)₂ . H₂O, PERMANGANATE METHOD			SAMPLE 2			FACTOR	SAMPLE 3		
				OBSERVED ROTATION, 200 MM. TUBE				OBSERVED ROTATION, 200 MM. TUBE		
	INDIVIDUAL	AVERAGE	CORRECTED	WITHOUT URANYL ACETATE	WITH URANYL ACETATE	DIFFERENCE		WITHOUT URANYL ACETATE	WITH URANYL ACETATE	DIFFERENCE
	per cent	per cent	per cent	°V.	°V.	°V.		°V.	°V.	°V.
Culter	46.25 45.64	45.95	45.07	4.2	14.53	10.33	4.39	2.3	12.57	10.27
Fisher	47.01 47.12	47.07	46.19	4.83	15.59	10.76	4.22	2.90	13.58	10.68
Haring	45.31 45.66 45.00	45.32	44.44	4.38	14.29	9.91	4.58	2.68	12.50	9.82
Reindollar	46.32 46.44	46.38	45.50	4.50	14.60	10.10	4.50	2.60	10.80	8.20
Shupe	46.63	46.63	45.75	3.7	11.6	7.9	5.75*	3.3	10.7	7.4
Average			45.39				4.42			

* Omitted from average.

of powdered uranyl acetate, stopper, shake 1 hour, cool to 20° C., make to volume with saturated uranyl acetate, filter, and polarize in a 200 mm. tube. Report reading in degrees Ventzke.

(3) Analyze 1 gram by the permanganate method [*This Journal,* 15, 457 (1932)].

The results of the collaborators are shown in Table 1. Rotations per gram per 100 cc. have been calculated on the basis of Sample 1 containing 86.39 per cent calcium gluconate monohydrate, the average of the results of all collaborators by the permanganate method.[1]

Sample 2 was directed to be analyzed by the permanganate method, and by the polarimetric method as outlined in the article of Fisher and Bailey,[2] with the exception that no factor was given for calculating percentage of calcium gluconate, the observed rotations only being requested. Sample 3 was to be analyzed by the polarimetric method only. Table 2 shows the results of the collaborators. The "corrected" figures for calcium gluconate by the permanganate method in Sample 2 are based on the fact that the cocoa and lactose present contained calcium equivalent to 0.88 per cent calcium gluconate. The "factor" was obtained by assuming Sample 2 to contain 45.39 per cent calcium gluconate (the average of the corrected permanganate values) and dividing this value by the difference between the observed rotations with and without uranyl acetate. It will be noted that the factors are in fair agreement with the exception of one result, and that the average factor, 4.4, is close to the factor 4.3 found by Fisher and Bailey. However, a similar calculation from the data of Table 1 yields a value for the factor of 4.0; this factor does not give the correct percentage of calcium gluconate when it is multiplied by the differences in rotation observed with Sample 2. The results with Sample 3 show so wide a variation that any calculations from them are useless.

The polarimetric method gave satisfactory results in the hands of the associate referee (within the limit of accuracy with which polarimetric readings could be made) both for pure calcium gluconate and for mixtures containing sugars, cocoa, and calcium lactate. The results obtained by the collaborators on this year's samples show, however, that the details of the method must be further studied before the method can be relied on to give correct results in the hands of an analyst using it for the first time. Samples containing cocoa yield rather dark-colored solutions whose rotations are difficult to read in the saccharimeter, particularly those solutions which are saturated with uranyl acetate. Two of the collaborators had to use a 100 mm. tube for some of their readings. Some means of further clarifying the solutions would be desirable. The question of the time required to shake the solutions with uranyl acetate in order to reach equilibrium should be further investigated. The associate referee

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[1] Determination of the calcium content of Sample 1 by the associate referee by conversion to calcium sulfate yielded an average value of 86.28 per cent calcium gluconate monohydrate.
[2] *This Journal,* 15, 465 (1932).

found one hour sufficient using the shaking machine in his laboratory, but more time might be required with other means of agitation. Dunbar and Bacon,[1] working with malic acid solutions, found two hours' shaking necessary to obtain the maximum rotation. These authors also found that sodium light gave lower readings than did white light. It is known that any marked acidity or alkalinity will affect the rotation of the uranium complex, and the acidity or alkalinity of the samples may have to be taken into consideration. The question of the effect of small temperature variations should be investigated. It would be desirable to have a number of analysts determine as accurately as possible the specific rotation of calcium gluconate and of the uranium complex, using a highly purified sample of anhydrous calcium gluconate.

RECOMMENDATIONS[2]

It is recommended that the polarimetric method for the determination of calcium gluconate be submitted to further collaborative study.

REPORT ON TETRACHLORETHYLENE

By FRED L. ELLIOTT[3] (U. S. Food and Drug Administration, Baltimore, Md.), *Associate Referee*

An attempt was made to determine the purity of tetrachlorethylene prepared for medical purposes by saponification with strong caustic potash in various solvents. No very satisfactory results were obtained, and therefore no samples were sent to collaborators.

The solvents used were alcohol, glycerine, amyl alcohol, xylene+amyl alcohol, and methyl alcohol. Sodium methylate was also used. The saponification was carried on in flasks fitted with reflex condensers and in pressure bottles heated on the steam bath. The degree of saponification was determined by evaporating off the solvent, acidifying with dilute nitric acid, and determining the chlorides.

The sample used conformed to the following degree of purity: no free chlorine, no chlorides, no weighable residue in 25 cc., slight odor of phosgene, boiling point = 120.4° C.

While the degree of saponification was not always uniform, tending to run low, approximately 70 per cent was obtained with alcohol and an average of 93.5 per cent with methyl alcohol and large excess of caustic potash.[4]

[1] *J. Ind. Eng. Chem.*, 3, 828 (1911).
[2] For report of Subcommittee B and action of the Association, see *This Journal*, 16, 54 (1933).
[3] Presented by T. F. Pappe.
    For report of Subcommittee B and action of the Association, see *This Journal*, 16, 54 (1933).

REPORT ON HEXYLRESORCINOL

By JONAS CAROL[1] (U. S. Food and Drug Administration, Chicago, Ill.),
Associate Referee

No method for the analysis of hexylresorcinol has been considered by this Association, and a review of the literature fails to disclose any methods.

Hexylresorcinol is a condensation product of caproic acid and resorcinol corresponding to the graphic formula—
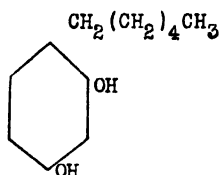
It is a white crystalline compound, melting point[2] 67.5–69.0° C., readily soluble in ether, chloroform, acetone alcohol, and vegetable oils; slightly soluble in petroleum ether; and very difficultly soluble in water. It is stated[3] that hexylresorcinol possesses marked germicidal properties, has a phenol coefficient of over 70, and is relatively nontoxic when administered by mouth.

For the work described hexylresorcinol was prepared in this laboratory according to the method of Dohme, Cox, and Miller.[2] As the crystals melted at 67.0–67.5° C., and were found to be free from intermediate compounds, the material was considered sufficiently pure for this investigation. Day and Taggart[4] applied the bromine method of Koppeschaar[5] to many phenolic compounds, including resorcinol and the cresols, but not to hexylresorcinol.

Preliminary study showed that time of reaction and initial temperature of bromination are influencing factors for complete bromination. Parts I and II of the table show that bromination is incomplete when the ordinary Koppeschaar procedure is used, even when the time of reaction is lengthened to overnight. Part III also shows incomplete reaction at elevated initial bromination temperature, but Part IV shows that complete bromination to dibromhexylresorcinol results when an initial temperature of 70° C. and an overnight reaction period are used.

Directions for the method and samples consisting of hexylresorcinol were sent to the collaborators.

[1] Presented by C. D. Wright.
[2] Dohme, Cox and Miller. *J. Am. Chem. Soc.*, 48, 1688 (1926).
[3] New and Nonofficial Remedies, p. 328 (1930).
[4] *J. Ind. Eng. Chem.*, 20, 545 (1928).
[5] *Z. anal. chem.*, 15, 233 (1876).

PERIOD OF BROMINATION	INITIAL TEMPERATURE	BROMINATION TO DIBROMHEXYLRESOR-CINOL
		per cent
I.—1 hour	25.0° C.	93.6
		93.9
		94.4
		93.5
		93.1
		93.8
II.—Overnight	25.0° C.	97.7
		97.3
		97.4
		96.6
III.—1 hour	70.0° C.	97.2
		97.5
		96.4
		95.3
IV.—Overnight	70.0° C.	100.2
		100.6
		100.3
		99.9
		99.7
		100.4
		99.7

HEXYLRESORCINOL

REAGENTS

(a) *Bromine solution.*—0.1 *N*. Dissolve 3 grams of potassium bromate and 50 grams of potassium bromide in water and dilute to 1 liter.

(b) *Sodium thiosulfate solution.*—0.1 *N*.

(c) *Sodium hydroxide solution.*—5 per cent.

(d) *Potassium iodide solution.*—20 per cent.

(e) *Hydrochloric Acid.*—Concentrated.

(f) *Chloroform.*

(g) *Starch solution.*

DETERMINATION

Dissolve about 0.05 gram of hexylresorcinol, accurately weighed, in 2 cc. of 5 per cent sodium hydroxide solution in a 250 cc. Erlenmeyer flask fitted with a glass stopper. Add 30 cc. of 0.1 *N* bromine solution. Heat on the water bath to about 70° C. Remove from bath, add 10 cc. of concentrated hydrochloric acid, and immediately insert the stopper. Shake for 15 minutes and allow to stand overnight. Remove stopper, add 5 cc. of potassium iodide solution, taking care no bromine vapors escape, and immediately stopper the flask. Shake thoroughly, wash stopper and neck of flask with a little distilled water, and add 1 cc. of chloroform. Titer quickly with 0.1 *N* sodium thiosulfate solution, adding starch indicator just before the end point is reached. 1 cc. of 0.1 *N* bromine solution = 0.004879 gram of hexylresorcinol.

Results of Collaborators.

COLLABORATOR	BROMINATION TO DIBROMHEXYLRESORCINOL
	per cent
F. C. Sinton,	100.7
U. S. Food & Drug. Adm.	100.4
New York	99.5
E. O. Haenni	100.2
U. S. Food & Drug Adm.	104.0
Cincinnati	99.0
	100.4
	103.0
M. L. Yakowitz	99.43
U. S. Food & Drug Adm.	98.79
San Francisco	98.16
	98.02
A. W. Hansen	97.6
U. S. Food & Drug Adm.	97.0
Minneapolis	
E. C. Deal	104.98
U. S. Food & Drug Adm.	107.44
New Orleans	103.56
	100.03

COMMENTS

E. C. Deal.—I would suggest that the analyst be instructed to titrate until a solution is obtained which remains colorless after being shaken briskly for about 30 seconds, as the chloroform and brominated hexylresorcinol tend to hold the iodine in solution.

E. O. Haenni.—I believe the method of analysis to be satisfactory after the analyst becomes accustomed to the titration.

F. C. Sinton.—In titrating quickly, as specified in the method, there was an apparent end point, but the iodine reappeared rapidly and a further titration of about 2 cc. of thiosulfate was obtained. This latter end point compared favorably with the usual iodine titration in permanency and was the one selected for the results which are reported.

DISCUSSION

While the theoretical quantity of hexylresorcinol in the sample was obtained in certain determinations, the results as a whole show considerable variation. This may be due (1) to the conditions of bromination, (2) to the end-point, and (3) to the weighing of the small sample as specified in the directions.

RECOMMENDATIONS[1]

It is recommended—

(1) That further study of the quantitative method be made to obtain more uniform results.

(2) That suitable qualitative tests for distinguishing hexylresorcinol from other phenolic compounds be studied.

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[1] For report of Subcommittee B and action of the Association, see *This Journal*, 16, 54 (1933).

REPORT ON ERGOT ALKALOIDS

By C. K. GLYCART[1] (U. S. Food and Drug Administration, Chicago, Ill.),
Associate Referee

Two recognized biological methods are available for the assay of ergot, viz., the cockscomb method official in the present U.S.P., and the Broom-Clark rabbit uterus method,[2] which requires the highly specialized technic of experienced pharmacologists.

According to the literature on the subject, investigators made comparatively little progress on quantitative chemical determinations of the alkaloidal content of ergot until 1930, when Smith[3] devised the colorimetric assay. Briefly outlined, the essential steps in the procedure are: (1) Extraction of alkaloids from ammoniacal solution with ether; (2) washing the ether free from alkaline soluble yellow pigments with ammoniacal water; (3) recovery of alkaloids from ether with dilute tartaric acid solution; (4) addition of para-dimethyl-amino-benzaldehyde H_2SO_4 reagent; and (5) microcolorimetric reading against a standard solution of ergotamine tartrate prepared in the same manner with reagent.

It is stated by Smith[3] that the reaction of para-dimethyl-amino-benzaldehyde in sulfuric acid with ergot alkaloids is photochemical, that a clear violet-blue color gradually develops at a rate depending on the intensity of the light, and that when developed to its maximum depth it is quantitatively proportional to the amount of alkaloid in the system. He concluded from his work that results obtained by the chemical method based on the application of this reaction to the ergot alkaloids individually, or in the fluidextract as observed by Smith and Stohlman[4], compare well with findings of the physiological method of Broom-Clark.

The sample submitted for study consisted of 5 cc. ampuls of standard No. 2160 fluidextract of ergot, the potency of which may have changed due to storage[5] at room temperature at Chicago after receipt from the pharmacological laboratory in Washington, D. C. The directions outlined from Smith's original method were sent to W. J. Rice, Eli Lilly & Co., Indianapolis, Ind., M. I. Smith, U. S. Public Health Service, Washington, D. C., and J. C. Munch, Bureau of Biological Survey, Washington, D. C.

CHEMICAL ASSAY FOR ALKALOIDS OF ERGOT BY MICROCOLORIMETRIC METHOD[6]

REAGENTS

(a) *Ethyl ether.*—U.S.P.

(b) *Ammonium hydroxide solution.*—10 per cent.

(c) *Tartaric acid solution.*—1 per cent.

(d) *Ergotamine tartrate.*

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[1] Presented by M. I. Smith.
[2] *J. Pharm.*, 22, 59 (1924).
[3] Public Health Reports, 45, 1466 (1930).
[4] *J. Pharmacol.*, 40, 77 (1930).
[5] *Ibid.*, 43, 621 (1931).
[6] *Proc. Am. Drug Mfrs. Assoc.*, p. 168 (1932).

(e) *Erlich-Smith reagent.* $\frac{M}{60}$ Para-dimethyl-amino-benzaldehyde in concentrated H_2SO_4. Dissolve 2.4856 grams in 1 liter of concentrated H_2SO_4.

Evaporate 5 cc. of the fluidextract on a water bath under a current of air or in vacuo to remove alcohol; avoid excess heating and transfer quantitatively with the aid of water to a separatory funnel. Add about 2 cc. of Reagent (b), or until distinctly alkaline to litmus paper. Extract with four successive portions of ether, using 40, 25, 20, 15 cc., or until alkaloids are removed completely. Combine the ether extractions in a separatory funnel, wash three times with about 25 cc. of water and a few drops of ammonium hydroxide to remove the yellow pigments. Finally wash twice with water to remove the excess of alkali. Shake the ether with an aqueous 1 per cent tartaric acid solution, using 10, 10, 10, and 5 cc. portions, respectively, or until alkaloids are removed completely. Evaporate on a water bath under an electric fan to remove the ether, transfer the solution to a 25 cc. volumetric flask, and make to volume. (Note: The solution may be diluted further if too concentrated for microcolorimetric determination.)

Prepare standard solutions containing 0.06, 0.08, and 0.10 mg., respectively, of ergotamine tartrate per 2 cc. of water.

Make the color comparisons in a series of five suitable small glass tubes (shell vials, 5 cm. high and 2 cm. in diameter with polished flat bottoms such as used in hydrogen-ion work are convenient).

Into the first of these tubes, transfer 1 cc. of the ergot-tartaric acid solution and add 1 cc. of water; into the second tube, transfer 2 cc. of the ergot solution; and into the other three tubes, place, respectively, 2 cc. of the three standard solutions. Add exactly 1 cc. of M/60 para-dimethyl-amino-benzaldehyde reagent from buret to the tubes, mix thoroughly, and place, if possible, in direct sunlight for 10 to 15 minutes or for a half hour to two hours or longer in diffused daylight of a cloudy day. When the color has developed to its maximum intensity of a clear violet-blue, read in a colorimeter.[1] Calculate the content of ergot alkaloids.

RESULTS AND COMMENTS

W. J. Rice.—Ampul was found to contain 0.0003325 gram of alkaloids in 1 cc., corresponding to 66.5 per cent of standard activity by microcolorimetric method.

We found it necessary to use more than the prescribed quantity of ether[2] in order to break up an emulsion that formed in the assay of both samples. It is our experience that a single washing with ammoniated water is not sufficient to entirely remove the yellow pigment.

M. I. Smith.—Colorimetric assay of the sample showed an activity of 0.34 mg. ergotamine tartrate per cc. Duplicate analysis yielded 0.33 mg. per cc.

A check by the method of Broom and Clark in three different experiments yielded the following:

1 cc. FE slightly greater than 0.3 mg. ergotamine tartrate.
1 cc. FE slightly less than 0.4 mg. ergotamine tartrate.
1 cc. FE nearly equal to 0.37 mg. ergotamine tartrate.

DISCUSSION

The results by the chemical method reported by Rice and Smith are in excellent agreement. The standard solutions of ergotamine tartrate were prepared independently. The checks by the Broom-Clark method reported by Smith are also in agreement. The method has been corrected

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[1] A Klett biocolorimeter with micro plungers and cups is suitable.
[1] U. S. Public Health Reports, **45**, 1466 (1930)

to include washing three times with ammoniated water to remove yellow pigments as specified in the original method and as pointed out by Rice in his comment.

Owing to the lack of facilities and time on the part of the associate referee this year the work was limited to the three collaborators, who have had wide experience regarding methods for ergot standardization.

It is recommended[1] that study of the chemical assay for alkaloids of ergot be continued.

M. I. Smith: The value of this or any other chemical method depends entirely upon whether or not it gives results which bear a definite relationship to those obtained by a recognized physiological method of assay. We have done quite a little work on this subject in the last two years. We have tested a large number of fluidextracts of ergot with a wide range of activity and found without exception very satisfactory agreement between the colorimetric method and the physiological method of Broom and Clark. Within the last year there appeared two or three reports on this subject from other laboratories in this country and I think on the whole they are quite favorable. One report appeared about a year ago from a Canadian laboratory, and this was distinctly unfavorable. I cannot go into a detailed discussion of that at this time. I particularly wish to call your attention to a report of the Subcommittee on Ergot of the British Pharmacopoeia Commission. This was published in October, 1931. This subcommittee made a collaborative study of several chemical methods, including the colorimetric method, and compared the results so obtained with the most reliable available physiological methods. I will read two or three of their conclusions:

1. That the colorimetric method carried out as recommended (essentially as I described it in 1930) permits an accurate estimation of total alkaloid in ergot and in liquid extract (fluidextract) of ergot.

2. That the colorimetric method has advantages over gravimetric methods in that smaller amounts of material suffice and the assay is more quickly completed.

3. That the biological methods are subject to a considerable margin of error and the evidence is that this margin is at least as great as that due to the variation in the relative proportion of ergotoxine and ergotinine in the total alkaloid.

May I say, in conclusion, that I fully agree with the recommendations of your associate referee as to the desirability of doing further collaborative work on this subject, if, in his judgment, further work is needed.

C. S. Leonard: Mr. Chairman, I wonder if we are not on the track of a way to avoid waiting for sunlight. We are using the ergotoxine not the fluidextract of ergot (because we don't put that out at all). We want to follow the effects of aging on some of our preparations and so we have been using the chemical method and our English colleagues have been

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[1] For report of Subcommittee B and action of the Association, see *This Journal*, 16, 54 (1933).

using the Broom and Clark method. In the case of "Ernutin," there is no question of the presence of the ergot alkaloid since the article is a solution of ergotoxine and tyramine. We have been able to follow the aging in this solution with the color reaction exactly as described by Dr. Smith. We discovered that the color could be developed with hydrogen peroxide. We did not have much hope of being able to develop it for ergot alkaloid. Hydrogen peroxide interferes. However, we tried it and found that at the right strength of hydrogen peroxide we could develop color in the dark and got perfect checks and if we got above that strength, we got an increasing rapidity of fading. It would fade in 24 hours. With strong peroxide we can understand why Dr. Smith said there was interference. If a proper strength is chosen, we get very good results. Also by using phosphoric acid instead of sulfuric acid, we got a pure blue. Colors develop in 10 minutes in the dark. Our British colleagues are going to try to check us on the thing. We have nothing to announce until we hear from them.

A. G. Murray: Both Dr. Smith and Dr. Leonard referred to the effect of the inert alkaloid but I am not clear as to whether or not the inert alkaloids develop this color in the same way as the active alkaloids do, so if anybody should ever succeed in separating the active from the inactive, would there be the problem of determining which is contained in any particular product? Are there any alkaloids other than the active ergot alkaloids that will yield this color?

M. I. Smith: There is an inactive isomer of ergotoxine, namely ergotinine; also an inactive isomer of ergotamine which is ergotaminine. Both these inactive isomers give the same color reaction as the physiologically active ones; as a matter of fact, from a practical standpoint, there should be no confusion about that, because it is very difficult to get either of these inactive isomers in any quantity from ergot and I believe that all the total alkaloids extracted from ergot by the process used in my method are practically physiologically active. There are no other substances that I know of that will give the same color reaction as the ergot alkaloids, with the exception of tryptophane and indol, and possibly skatol. These substances, however, even if present in the fluidextract of ergot, will not be extracted by the method used for the extraction of the alkaloids. The British workers believe that the physiologically active alkaloids of ergot bear a constant relation of some 70 to 80 per cent to the total alkaloids, so that if you measure accurately the total alkaloids of ergot you have a reasonably accurate index of the physiological activity of the fluidextract.

A. G. Murray: It is reported by pharmacologists that fluidextract of ergot gradually deteriorates. Is that deterioration due to the going over of the active alkaloids into the inactive ones and would this chemical colorimetric test show the alkaloids still as inactive as ever?

M. I. Smith: This is a very good point. We have studied the problem of deterioration of ergot alkaloids in the fluidextract of ergot under a variety of conditions and have uniformly found a good agreement in the chemical and physiological methods of assay as applied to preparations which have deteriorated all the way down to zero activity.

REPORT ON MICROCHEMICAL METHODS FOR SYNTHETICS

By Irwin S. Shupe (U. S. Food and Drug Administration, Chicago, Ill.),
Associate Referee

No microchemical methods for synthetics have been adopted by this Association and publications on this subject are not numerous. Preliminary work included a search for suitable tests by applying 30 reagents usually employed in microchemical work to the following synthetics: Barbital and phenobarbital (Van Itallie and Steenhauer[1] and Deniges[2]), cinchophen, benzocaine, chinosol, pyridium, acetanilide, antipyrine, amidopyrine, phenacetin, hexamethylenetetramine, stovaine, novocaine, dibromin, phenol, resorcinol, salicylic acid, benzoic acid.

Benzocaine, chinosol, cinchophen, and pyridium were chosen for study this year. The microchemical reactions of the four synthetics with 16 reagents (Stephenson[3]) are given in the following table.

Crystalline precipitates are indicated by (c); Non-crystalline by (a); No reaction by (−).

	GOLD CHLORIDE*	PLATINIC CHLORIDE*	WAGNER'S†	MAYER'S (U.S.P.)	MARME'S†	KRAUT'S†	BROMINE WATER (SAT.)	KSCN*	NaOH*	Na₂CO₃*	Na₂H PO₄*	SODIUM BENZOATE*	PICRIC ACID (SAT. WATER SOLN.)	POTASS. DICHROMATE*	POTASS. FERROCYANIDE*	MAGNESIA MIXTURE†
benzocaine 1:100	a	c	c	—	—	c	—	—	c	c	c	c	c	—	c	c
chinosol 1:500	—	—	c	c	—	a	c	—	—	—	—	—	c	—	—	c
cinchophen 1:1000	c	—	—	—	—	a	a	—	—	—	—	—	a	—	—	—
pyridium 1:1000	c	a	c	c	c	a	a	c	c	c	c	a	a	c	c	c

* Consists of approximately 5 per cent solution in water.
† *Methods of Analysis, A.O.A.C.*, 1930.

[1] Microchemie, Emich Festschrift, p. 166–69 (1930).
[2] *Microchemie, 9,* 316 (1931).
[3] Some Microchemical Tests for Alkaloids (1921).

Ten crystalline precipitates were formed with benzocaine. Potassium ferrocyanide was selected as the best reagent. Five crystalline precipitates were formed with chinosol. Magnesia mixture produced the most sensitive and characteristic test. One reagent, gold chloride, yielded characteristic crystals with cinchophen. Eleven crystalline precipitates were formed with pyridium. Potassium thiocyanate produced the most sensitive and characteristic test.

The materials used for study were tested for purity and compared with their respective standards. Benzocaine and cinchophen were recrystallized products complying with U.S.P. requirements. Chinosol, a brand of oxy-quinoline sulfate complied with standards set forth in New and Non-Official Remedies (1930). Pyridium, a brand of phenylazo-alpha-alpha diamino pyridine hydrochloride, complied with standards suggested in an article by Collins.[1] These products were considered satisfactory for this work.

Directions for tests, control specimens, and unknown samples for identification were sent to the collaborators. The unknown samples consisted of the following:

I. Approximately 1:500 solution of chinosol colored with methyl red indicator.
II. Powdered benzocaine.
III. Approximately 1:1000 solution of pyridium.
IV. Powdered cinchophen.

The method has been published.[2]

RESULTS OF COLLABORATORS

H. McCausland, Abbott Laboratories, North Chicago.—

	Reagent	Description of crystals
I. Chinosol	Magnesia mixture	Small, yellow, elliptical crystals
II. Benzocaine	Potass. ferrocyanide	Large, irregular, colorless rods
III. Pyridium	Potass. thiocyanate	Small, dense, red-brown rosettes
IV. Cinchophen	Gold chloride	Clusters of minute yellowish crystals of indefinite form.

H. R. Bond, U. S. Food & Drug Adm., Chicago.—I.—Chinosol; II.—Benzocaine; III.—Pyridium; IV.—Cinchophen.

E. O. Eaton, U. S. Food & Drug Adm., San Francisco, Calif.—I.—Chinosol; II.—Benzocaine; III.—Pyridium; IV.—Cinchophen.

However, the description of the microcrystalline precipitates are of doubtful usefulness, especially if you were working on an absolute unknown.

I should prefer to use photographs, similar to ones used by Stephenson in his scheme, "Some Microchemical Tests for Alkaloids" as word descriptions are seldom adequate.

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[1] *J. Am. Pharm. Assoc.*, May, 1931.
[2] *This Journal*, 16, 84 (1933).

Wm. J. McCarthy, U. S. Food & Drug Adm., Cincinnati.—

Sample	Identification	Remarks
I.	Chinosol	Small elliptical grains
II.	Benzocaine	Colorless irregular plates and rods with potassium ferro-cyanide. Small rhombic crystals and few small plates with magnesia mixture. Solution of 1 to 100 in HCl
III.	Pyridium	Reddish brown, dense sheaves about two times larger than synthetic
IV.	Cinchophen	Dark clusters of needles. Solution of 1 to 1000—dissolved in 0.1 N NaOH and then made faintly acid with dilute HCl

*Frank C. Sinton, U. S. Food & Drug Adm., New York.—*I.—Chinosol; II.—Benzocaine; III.—Pyridium; IV.—Cinchophen.

Cinchophen and chinosol crystals were observed essentially as described.

Benzocaine, large, colorless, overlapping, irregular plates and large rods.

Pyridium, with potassium thiocyanate—small red brown sheaves. Also large burrs of fine needles.

No difficulty was experienced in identifying the crystals from the descriptions using controls.

M. R. Miller, Agricultural Experiment Station, Reno, Nevada.—

Sample	Identification	Comments
I.	Chinosol	(With magnesia mixture): Obtained the small elliptical grains and burr-shaped crystals: not immediately formed but developed after a half minute or more.
II.	Benzocaine	(With potassium ferrocyanide reagent): Colorless plates and rods. Observed also H-shaped twins.
III.	Pyridium	(With potassium thiocyanate reagent): Gave a few dense clusters of fine, red-brown needles. The concentration of pyridium in this sample seemed to be less than 1:1000 and the clusters of needles appeared more slowly than with a known 1:1000 solution of pyridium.
IV.	Cinchophen	(With gold chloride reagent): Dark clusters of needles, but failed to obtain short rhombic crystals in either this unknown or the known solution.

G. M. Johnson, U. S. Food & Drug Administration, Chicago.—

Synthetic		Reagent	Description of Crystals
I.	Chinosol	Magnesia mixture	Oval masses. A few needles
II.	Benzocaine	Potassium ferrocyanide	Long prisms at different angles often in groups
III.	Pyridium	Potassium thiocyanate	Irregular, almost circular masses with projecting spines
IV.	Cinchophen	Gold chloride	Needles of fragmented prisms

James H. Cannon, U. S. Food & Drug Adm., Chicago.—

Sample	Found	Remarks
I.	Chinosol	Seems highly characteristic
II.	Benzocaine	Crystallization satisfactory
III.	Pyridium	Crystallization satisfactory
IV.	Cinchophen	Crystallization satisfactory

Good crystals answering the descriptions given were obtained without difficulty in all cases.

DISCUSSION

The unknown samples were correctly identified by each of the collaborators. The microchemical tests for benzocaine, cinchophen, chinosol and pyridium are considered satisfactory. Eaton in his comments suggests the use of photomicrographs.

RECOMMENDATIONS[1]

It is recommended—

(1) That the microchemical methods for benzocaine, cinchophen, chinosol, and pyridium be adopted as tentative.

(2) That further study be made of microchemical methods for the more important synthetics.

No report on biological testing was given by the associate referee.

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[1] For report of Subcommittee B and action of the Association, see *This Journal*, 16, 54 (1933).

CONTRIBUTED PAPERS

NOTE ON DETERMINATION OF HYDROGEN PEROXIDE[*]

By A. K. BALLS and W. S. HALE (Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D.C.)

In the estimation of hydrogen peroxide, the iodometric method has the advantage over the more usual permanganate titration in that it is uninfluenced by sugars, glycerin, and like substances. Even phenols, including the actively reducing pyrogallol, react so slowly with iodine and with hydrogen peroxide that they are ineffective. In general, the ballast material met with in the analysis of agricultural products makes no appreciable change in the iodometric results; with permanganate, on the other hand, the peroxide determination in the presence of such substances is impossible.

The iodometric method, however, particularly with small quantities of hydrogen peroxide, has also a disadvantage: The quantity of iodine liberated from potassium iodide by hydrogen peroxide in acid solution slowly increases as the mixture stands. The amount of this increase is small, and with concentrated solutions would pass unnoticed. With small quantities of peroxide, however, it may form a substantial part of the total titration and lead to results far in excess of the amount of hydrogen peroxide actually present. The reaction by which this excess of iodine is liberated is not definitely known, but the presence of iodate in the iodide does not seem to explain it.

The use of molybdic acid as a catalyst speeds up the liberation of iodine by peroxide, without greatly affecting the subsidiary decomposition of the iodide due to other causes. By titrating the liberated iodine shortly after mixing the reagents it is possible with the catalyst to obtain a result representing the peroxide added, plus only a very small error due to the subsidiary iodine liberation, as this has gone on for only a short time. The method is accurate, and it has been used, for example, in determining the activity of the enzyme catalase[1,2] where the concentration of hydrogen peroxide is small. Nevertheless, the time allowed for the liberation of iodine is important, and it must be so selected that as nearly as possible the correct quantity of iodine is titrated.

Although for the reasons stated the iodometric method without a heavy metal gives less satisfactory results, it is impossible to use such a catalyst in the presence of phenolic reducing substances. Even in the strongly acid solutions used, pyrogallol, for example, is oxidized by the peroxide, which is therefore used up before it liberates iodine. The action of the metal catalyst therefore resembles that of a peroxidase, as A. Bach[3] has pointed out.

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[1] K. G. Stern. *Z. physiol. Chem.*, **204**, 259 (1932).
[2] A. K. Balls and W. S. Hale. *This Journal*, **15**, 483 (1932).
[3] *Ber.*, **65**, 1788 (1932).

While studying the estimation of hydrogen peroxide in the presence of peroxidase and its substrates (all reducing substances) the writers observed that the presence of considerable pyrogallol in the acid-iodide-peroxide mixture results in an action opposite to that of the metal catalyst. It appears to slow down only slightly the liberation of iodine equivalent to the added peroxide, but to materially retard the formation of any excess iodine Quantitative results may therefore be secured in this manner, apparently for the reason opposite to that obtaining in the presence of molybdic acid. Ordinary phenol acts as does pyrogallol, but not to an extent sufficient to give quantitative results.

The addition of pyrogallol in the iodometric titration is of advantage only when reducing substances of the same type are present in sufficient quantity to invalidate both the permanganate and the usual iodometric titrations, yet not in sufficient quantity to fill the role of the pyrogallol as described. To the authors the method is of importance because it permits the estimation of peroxide in the presence of small quantities of pyrogallol itself, but there are many agricultural products in the analysis of which the addition of the pyrogallol may at least increase the "factor of safety."

The determination of hydrogen peroxide in the presence of reducing substances, such as pyrogallol, has not heretofore been successful. By the scheme described above, it is easily made.

EXPERIMENTAL

Solutions of pure hydrogen peroxide, standardized against potassium permanganate, were also analyzed as follows: 20 cc. of peroxide solution was added to 25 cc. of 2 N sulfuric acid containing pyrogallol, as shown in the table. Thereafter, 10 cc. of 10 per cent potassium iodide solution was added, and the resulting iodine was titrated with appropriate thiosulfate solution (0.01 N or 0.1 N) after the time intervals stated below.

It will be noted that the best results were obtained under these conditions with about 0.5 gram of pyrogallol per titration set-up, and in a time interval of between 8 and 30 minutes. The method is specially adapted to small quantitites of peroxide, for with small quantities of liberated iodine no reduction of the iodine by the pyrogallol takes place for one or two hours. This is not the case, however, with greater iodine concentrations.

The oxidation products formed by oxygen in alkaline pyrogallol solutions are also capable of liberating iodine from hydriodic acid. They, therefore, behave as peroxides and must be absent from the pyrogallol used. On the other hand purpurogallin, the product of pyrogallol oxidation with hydrogen peroxide,[1] does not liberate iodine nor does it otherwise interfere with the reaction. The iodometric titration of hydrogen peroxide therefore forms a basis for a satisfactory method of measuring peroxidase action on substances such as the polyhydric phenols.

[1] R. Willstätter and H. Heiss. *Ann.*, **433**, 17 (1923).

Mg.H₂O₂ USED (DETERMINED BY K₂Mn₂O₈)	TIME OF STAND-ING	NO ADDITIONS		PYROGALLOL 0.05 g.			PYROGALLOL 0.10 g.			PYROGALLOL 0.20 g.			PYROGALLOL 0.50 g.				PHENOL	
		0.75	21.05	0.19	0.38	0.75	0.19	0.38	0.75	0.19	0.49	21.05	0.19	0.38	0.75	21.05	0.2 g.	0.5 g.
Mg.H₂O₂ found iodometrically (after standing)	4′	0.65	19.30			0.62	0.18	0.31	0.58	0.19	0.47	19.1	0.18	0.36	0.64	19.2	0.75	0.75
	5′	0.72									0.49						0.71	
	8′	0.80	21.02			0.75	0.20	0.37	0.72	0.20	0.49	20.2	0.19	0.38	0.73	20.6	0.77	0.75
	10′	0.79		0.20	0.39						0.49							
	12′	0.80	21.50	0.20	0.39	0.77	0.20	0.38	0.75	0.20		21.1	0.20	0.38	0.75	20.7		0.78
	20′		21.50			0.79	0.21	0.38	0.77	0.22		21.0	0.20	0.39	0.76	20.3		0.78
	30′		21.30			0.80				0.25		21.1	0.21	0.39	0.75	19.7	0.79	0.80
	60′	0.84																
	120′		21.40			0.82			0.80	0.28		19.5	0.23	0.43	0.73	16.0	1.06	0.85

SOURCES OF ERROR IN THE GUTZEIT METHOD FOR THE DETERMINATION OF ARSENIC

By C. R. GROSS (Insecticide Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.)

A revision of the Gutzeit method for the determination of arsenic applicable to foods and plants was adopted as official in 1930 and was incorporated in the 3rd edition (1930) of *Methods of Analysis, A.O.A.C.* Although the revision improved the method in regard to accuracy and convenience of operation, it was later found by the writer[1] that under certain conditions low results are obtained through interference caused by undigested pyridine residues when the method is used to analyze tobacco (nicotine contains pyridine ring) or other materials containing pyridine compounds. It was also reported to the A.O.A.C. by H. Heidenhain early in 1932 that low results might follow from incomplete reduction of the arsenic when large aliquots are subjected to the A.O.A.C. method of "cold" reduction. The results of investigations of these sources of error are given in this paper.

INTERFERENCE OF UNDIGESTED PYRIDINE RESIDUES

In the publication cited above the writer gave the results of an investigation showing that the Gutzeit method gives low yields when used for the determination of arsenic in tobacco, owing to undigested pyridine residues from nicotine which considerably retard the evolution of hydrogen and arsine during analysis. It was also found that all pyridine compounds interfere to a greater or lesser degree even after a very thorough acid digestion.

Fortunately, however, few products analyzed for arsenic, aside from tobacco, are likely to contain sufficient pyridine rings or its derivatives to cause low results. Among the possibilities may be mentioned apples or other agricultural products sprayed late in the season with nicotine sulfate following earlier spraying with an arsenical insecticide. In these, or in other cases where the presence of pyridine compounds is suspected in the product, steps must be taken to eliminate this source of error.

The most convenient method developed by the writer to eliminate the interfering compounds makes use of the A.O.A.C. procedure[2] for the treatment of coal-tar food colors preliminary to analysis by the Gutzeit method. By means of this method, the arsenic is precipitated along with the phosphate by treatment of the digested solution with ammonium hydroxide, phosphoric acid, and magnesia mixture. The precipitate containing the arsenic can then be filtered and washed free of the soluble pyridine residues, redissolved in a hydrochloric acid solution, and an-

[1] *Ind. Eng. Chem. Anal. Ed.*, **5**, 58 (1933).
[2] *Methods of Analysis, A.O.A.C.*, 1930, 203.

alyzed by the Gutzeit method. The details of the modified method are given in the same publication.

INCOMPLETE REDUCTION OF ARSENIC

Owing to the slow rate at which pentavalent arsenic is reduced to arsine by nascent hydrogen, the arsenic in the solutions being analyzed must be quantitatively reduced to the trivalent form before the hydrogen evolution is begun. The revised Gutzeit method specifies potassium iodide and stannous chloride as reducing agents and also a reduction period of 10–15 minutes at 20–25°C. Tests made with this "cold" reduction procedure previous to the revision of the Gutzeit method had indicated it to be satisfactory, therefore it was adopted as official in place of the less convenient method of hot reduction (heating to 90°C.) used formerly.

At the request of H. J. Wichmann, General Referee on Metals in Foods, the writer made a thorough investigation of Heidenhain's criticisms of the cold reduction method. Before the experimental work is described, the factors involved in the reduction may be briefly discussed.

The time required to reduce quantitatively pentavalent arsenic to the trivalent form depends on the temperature of the solution and the concentration of the reducing agents (in this case governed by the size of the aliquot selected for analysis). The lower the temperature and the larger the aliquot, the longer will be the time required.

The following experiment illustrates these facts and shows (1) that incomplete reduction by the A.O.A.C. cold procedure occurs only when large aliquots (20–30 cc.) are used and (2) that reduction is less complete at 20° than at 25°C.

Procedure.—Three accurately weighed portions of pure lead arsenate were digested with nitric and sulfuric acids and diluted in such manner that 10 cc. of the first solution, 20 cc. of the second, and 30 cc. of the third contained pentavalent arsenic equivalent to 30 micrograms of As_2O_3. Each solution contained approximately 18 cc. of sulfuric acid per liter. Three standard As_2O_3 solutions were prepared to contain the same concentrations of arsenic and sulfuric acid as above.

One series of cold reduction tests was made on these solutions at 20° C. and another at 25° C. In each case, duplicate 30, 20, and 10 cc. aliquots of the digested lead arsenate solutions (each aliquot representing 30 micrograms of As_2O_3) and corresponding aliquots of the standard As_2O_3 solutions were reduced for periods of 15, 30, and 60 minutes. Previous to use, all solutions had been adjusted to the proper reduction temperature, and a constant temperature bath was used to maintain this temperature during reduction.

A series of hot reduction tests was made at the same time for purposes of comparison. In these tests, duplicate 30 cc. aliquots of both digested lead arsenate solution and standard As_2O_3 solution (each aliquot repre-

TABLE 1

Influence of size of aliquot and length of reduction period upon completeness of reduction at 20° and 25°C.
(All aliquots contained arsenic equivalent to 30 micrograms of As_2O_3.)

RUN NO.	SOURCE OF ARSENIC	ALIQUOT	AVERAGE LENGTH OF 2 STAINS (MM.)						"HOT" REDUCTION*	
			REDUCTION PERIOD							
			15 MIN.		30 MIN.		60 MIN.			
			A	B	A	B	A	B	A	B
		cc.								
1	As_2O_3	30	20.5	1	21.0	1	21.2	1	21.0	1
1	PbHAsO₄	30	17.5	4	19.8	2	21.2	1	21.5	1
					"Cold" Reduction at 20°C.					
2	As_2O_3	20	21.2	1	20.8	1	20.5	1	21.5	1
2	PbHAsO₄	20	20.8	2	22.0	1	21.1	1	21.8	1
3	As_2O_3	10	22.5	1	23.0	1	23.2	1	23.5	1
3	PbHAsO₄	10	22.2	1	23.0	1	22.5	1	23.0	1
					"Cold" Reduction at 25°C.					
4	As_2O_3	30	21.0	1	20.8	1	20.8	1	21.2	1
4	PbHAsO₄	30	18.0	2	20.8	1	20.5	1	20.5	1
5	As_2O_3	20	17.5	1	—	—	—	—	17.2	1
5	PbHAsO₄	20	17.8	1	—	—	—	—	17.8	1
6	As_2O_3	10	17.2	1	—	—	—	—	17.8	1
6	PbHAsO₄	10	17.2	1	—	—	—	—	17.2	1

* 30 cc. aliquots used throughout.

senting 30 micrograms of As_2O_3) were reduced by adding 5 cc. of 15 per cent potassium iodide solution and 5 cc. of hydrochloric acid, heating to 90°C., adding four drops of 40 per cent solution of stannous chloride in hydrochloric acid and heating for 10 minutes. The solutions were then cooled to the proper evolution temperature.

At the end of the cold and hot reduction periods, the contents of each bottle were diluted to 40 cc., 2 grams of 30-mesh zinc was added, the upper tubes of the apparatus were connected, and evolution was allowed to proceed for 1½ hours at the same temperature as that employed for cold reduction. The reductions and the evolutions were made in runs of 16 bottles each (12 cold reductions and 4 hot). This afforded the advantage of employing a single complete sheet of Hanford-Pratt strips for each run.

At the end of the first evolution period, the strips were removed, fresh strips were introduced, fresh portions of zinc and the hydrochloric acid-stannous chloride reagent were added, and the evolution was continued for a second period of 1½ hours to determine the residual arsenic remaining in the solutions. Separate tests showed that no further reduction was necessary for this determination as the arsenic, though not always completely reduced to the trivalent form at the beginning, was always completely reduced at the end of the first period of evolution.

Results.—The way in which the runs were grouped and the lengths of the stains resulting from the tests are shown in Table 1. The "A" columns in this table give the average stain lengths after the first period of evolution and the "B" columns give the lengths of residual stains developed during the second period. Residual stains of 1 mm. are due to arsenical impurities contained in the reagents.

Incomplete reduction and evolution of arsenic from the lead arsenate solutions during the first period is indicated wherever stains exceeding 1 mm. in length are formed during the second period of evolution. Reference to the table shows that in every case of this kind the stains during the first period are shorter than the corresponding standard As_2O_3 stains. It should be noted that these examples of incomplete reduction occur only when large aliquots are used (20–30 cc. at 20° C. and 30 cc. at 25° C.) and that reduction of even 30 cc. aliquots is complete after 30 minutes at 25° C. and nearly so after 30 minutes at 20° C.

The stains obtained in the cases of incomplete reduction cited were evaluated by comparison with standard stains from the same run. The results, expressed as percentage of arsenic recovered, are shown as follows:

ALIQUOT	REDUCTION TEMPERATURE	TOTAL ARSENIC RECOVERED AFTER REDUCTION FOR—	
		15 MIN.	39 MIN.
cc.	*°C.*	*per cent*	*per cent*
30	20	76.7	95.0
20	20	95.0	—
30	25	80.0	—

Tests were then made to determine whether results similar to the above would be obtained when aliquots representing 10 to 30 micrograms of As_2O_3 and stick zinc were used.

FIGURE 1

VOLUMES OF HYDROGEN EVOLVED BY 30-MESH AND STICK ZINC DURING 1 1/2 HOURS AT 20° C.

——— 30-mesh zinc
— — — stick zinc

PERIOD OF EVOLUTION (MINUTES)
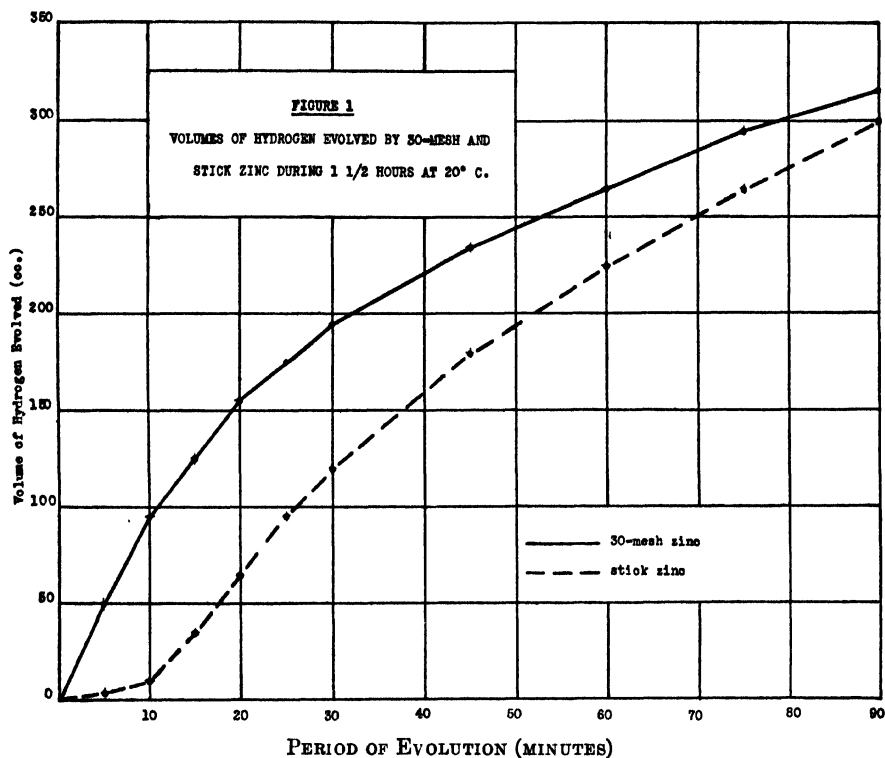
Procedure.—A series of six Gutzeit runs (three with 30-mesh zinc and three with stick zinc from the same manufacturer) was made. Each run was composed of duplicate determinations carried on under each of the following conditions.

ARSENIC SOLUTION USED	ARSENIC AS As_2O_3 PER ALIQUOT (MICROGRAMS)
Standard As_2O_3	10
Digested PbHAsO₄	10
Standard As_2O_3	30
Digested PbHAsO₄	30

All aliquots were diluted to 30 cc. before reduction, and reduction was carried on for 15 minutes at 20° C. (In the previous test, this combination of conditions had resulted in a yield of 76.7 per cent of the arsenic.) Thirty-mesh zinc was used at the rate of 2 grams per generator bottle and stick zinc at the rate of five pieces, total weight approximately 16 grams.

Results.—The results, calculated as percentage of arsenic recovered, are shown in the following table:

RUN NOS.	TYPE OF ZINC	AVERAGE PERCENTAGE OF ARSENIC RECOVERED MICROGRAMS OF As_2O_3 PER ALIQUOT—	
		10	30
1, 2, 3,	30-mesh	75.7	77.3
4, 5, 6	stick	93.3	92.7

These results show approximately the same recoveries of arsenic when 10 and 30 microgram aliquots are used under the same conditions of reduction and evolution. However, when stick zinc is used in place of 30-mesh zinc distinctly higher recoveries are obtained. The explanation for this difference is found in Fig. 1, which shows the relative rate at which hydrogen is generated by these two forms of zinc during evolution at 20° C. It will be noted that in the case of stick zinc there is very little hydrogen generated during the first 10 minutes, while with 30-mesh zinc it starts off at a very high rate. After 15 minutes, only 35 cc. (12 per cent of the total evolved after 1½ hours) has been generated by stick zinc, whereas with 30-mesh zinc, 125 cc. (40 per cent of the total) has been evolved. The slow rate at which evolution starts with stick zinc allows the arsenic additional time in which to become reduced to As_2O_3 before much of the total hydrogen has been evolved.

All the tests given show that complete reduction can be obtained by specifying in the present A.O.A.C. procedure a reduction period of at least 30 minutes at not less than 25° C. If a shorter reduction period is desired, it is suggested that tests be made to determine the minimum temperature to which the solutions may be warmed in order to insure complete reduction in the time chosen.

POLARIMETRIC METHOD FOR ESTIMATION OF THE SACCHAROGENIC POWER OF FLOUR[1]

By H. C. Gore (The Fleischmann Laboratories, Standard Brands Incorporated, New York, N. Y.)

There are at least four well-defined manifestations of diastatic power in the dough batch acting concurrently on the starch, namely (1) starch hydration, or the change of the degree of hydration of the starch; (2) saccharogenesis, or the attack of diastase on the raw starch forming maltose and other copper-reducing substances; (3) liquefaction, or the liquefying of the cooked starch paste to form soluble starch; and (4) saccharification, or the transformation of the dissolved soluble starch into reducing sugars and dextrin.

Starch hydration is evidenced by the incipient disintegration of the starch granule in the dough on baking whereby the starch crumb becomes more susceptible to the action of flour diastase, and the crumb stales less rapidly than crumb in which the starch has been subjected to less diastatic action. It is usually measured by various methods for recording the rate of staling applied to the crumb of the loaf. The greater the diastatic action on the starch grain, the slower the rate of staling. These methods were recently reviewed by Schultz and Landis.[1] More rapid and possibly more convenient methods are those of Platt[2] and Bailey.[3] Schultz and Landis measured the increase in the formation of sugars in the crumb of the loaf by the action of flour diastase, using a fermentation method. They found that the greater the degree of hydration of the starch, the slower the rate of staling and the greater the action of the flour diastase on the starch.

The saccharogenic action is measured by determining the increase in copper reduction in a dough or flour suspension under standard conditions. A well-known method is that of Rumsey.[4] In a series of ten bread flours analyzed by the writer and Dr. Jozsa,[5] the average saccharogenic value by the Rumsey method was 1.2. Thus 1.2 grams of reducing substances as maltose was formed from 100 grams of flour per hour under the conditions of the method.

Liquefaction is conveniently measured by the method of Jozsa and Gore.[6] One degree liquefying power is defined as the ability of the flour to liquefy its own weight of starch under the conditions of the method. The average liquefying power of the ten wheat flours examined was 1.1.

In case of the saccharifying value (Lintner value) a malt infusion was defined by Lintner as having a value of 100 when one-tenth of 1 cc. of a 5 per cent infusion produces enough reducing substances in 10 cc. of a 2 per cent solution of soluble starch in 1 hour at 21° C. exactly to reduce all the copper in 5 cc. of Fehling's solution.

Gore and Jozsa also have shown that if the digestion of the soluble starch in Lintner's method takes place at 4.8 pH, 100° Lintner is equivalent to the production of 7.03 grams of reducing sugars, reckoned as maltose, by 1 gram of sample. The ten flours had Lintner values averaging 88°, measured by determining the activity of filtered suspensions, the suspensions containing a little added salt to promote the solubility of the enzyme. This value accordingly was equal to the formation of 620 grams of reducing sugars from 100 grams of flour.

Thus, under the conditions of the Rumsey saccharogenic diastase method, 1.2 per cent of reducing substances calculated as maltose is

[1] *Cereal Chem.*, 9, 305 (1932).
[2] *Ibid.*, 7, 1 (1930).
[3] *Ibid.*, 9, 65 (1932).
[4] *Am. Inst. Baking Bull.* 8 (1922).
[5] *Ind. Eng. Chem.*, 24, 99 (1932).
[6] *Ibid.*, 95.

liberated from 100 grams of flour (pH of the 1–10 flour suspension, 5.4 to 5.6, 1 hour digestion at 27° C.); under the condition of the liquefying method 111 grams of starch is liquefied by 100 grams of flour (pH 4.6, 1 hour digestion at 21° C.); and under the condition of the Lintner method 620 grams of reducing substances reckoned as maltose is produced by the diastase in 100 grams of flour (pH 4.6, 1 hour digestion at 21° C.). It is true that these activities are measured at different temperatures (27°, 21°, and 21° C., respectively) and at different pH values (pH of flour suspension approximately 5.4 to 5.6, 4.6, and 4.6, respectively), so that they are not strictly comparable. It is evident, however, that flour is endowed with liquefying and saccharifying activities greatly in excess of the saccharogenic power. Saccharogenesis is thus by far the slowest of the reactions in the train of events attending the dissolution of the starch. It thus becomes the most important of these manifestations of diastase in flour.

Many modifications of the Rumsey method have been proposed. Malloch[1] used a citrate buffer giving the flour suspension at pH of 4.7. Blish, Sanstedt and Astleford[2] used the same buffer and digested at 30° C. They recommended a picric acid method for estimating the reducing substances present, and considered that the blank in a normal flour was so small that it could be neglected.

Berliner and Rüter[3] digested the 1–10 suspension for ½ hour at 25° C. and estimated the reducing substances by a slight modification of the Lane-Eynon[4] method, the filtered flour extract being added to the hot Fehling solution in amounts of 1–1½ cc. at a time instead of dropwise. These authors also polarized their flour filtrates and presented extensive data showing the relation between the reducing substance increases and the polarization increases. Polarization was found superior to the Lane-Eynon method in simplicity and speed. Occasionally however turbid solutions were encountered. They used their modification of the Rumsey method and the polarization increases in a series of studies on hard wheat flour (Manitoba) and soft wheat flour (Swedish) and on starches prepared therefrom, with and without added diastase preparation at the end of different times of digestion up to total periods of 6 hours. The polarization increases in general paralleled the increases in reducing sugar.

Pelshenke[5] measured saccharogenesis in flour by autolysing 13.3 per cent suspensions (4 grams of flour and 30 cc. of water) at 32° C. for ½ hour. He found that production of turbid filtrates could be avoided by adding a few drops of toluene at the beginning of the digestion. All of these authors terminated the digestion by adding sodium tungstate and sulfuric

[1] *Cereal Chem.*, **6**, 175 (1928).
[2] *Ibid.*, **9**, 378 (1932).
[3] *Z. ges. Mühlenw.*, **5**, 116, 134, 156 (1928); **7**, 63 (1930).
[4] *Methods of Analysis*, A.O.A.C., 1930, 377.
[5] *Mühlenlab.*, **5**, 37 (1931).

acid substantially in the manner described by Rumsey and then centrifugalized, or filtered.

The data of Berliner and Rüter[1] showed that in flour the starch is not uniform in regard to its susceptibility to diastase, but that part of it is very rapidly converted into reducing substances.

The primary object of the work described in this paper was to develop a method for measuring the saccharogenesis of a flour, so that a single polarization of a flour filtrate, made after the autolysis of a flour suspension, would correctly define the relative saccharogenic activity in terms of reducing substances formed, reckoned as maltose. The lack of uniformity of the "attackability" of the flour starch made it difficult to determine the conditions for the autolysis. An idea of the nature of the problem is shown by the curves in Chart 1. Here the polarization of successive samples of the filtrates of five flour suspensions is shown after incubation at 27° C. at different intervals up to a total time of 7 hours. The method used was as follows:

Twenty-five grams of the flour sample was put into a 500 cc. Erlenmeyer flask, and 241.5 cc. of water at 27° C. was added. After thorough mixing the suspension was incubated in a water bath at 27° C., with frequent mixing, for the given time. Then 7.5 cc. of sodium tungstate (15 grams per 100 cc.) was added and well mixed, and 1 cc. of concentrated sulfuric acid was added with thorough mixing. The suspension was then immediately filtered on a folded filter and the polarization measured at room temperature (about 25° C.) within half an hour from the time the sulfuric acid was added.

The check non-incubated sample was prepared by mixing 25 grams of the flour with a mixture of 241.5 cc. of water, 7.5 cc. of the sodium tungstate solution and 1 cc. of sulfuric acid, and filtering and polarizing in the manner above described. The polarization of 1–10 flour suspension filtrates at 27° C. (degrees Ventzke, 4 dcm. tube) follows:

FLOUR	INTERVALS, HOURS						
	0	¼	½	1	2	4	7
Kansas	−0.1	1.3	1.45	2.1	2.6	3.8	4.9
Texas	±0.0	1.2	1.40	1.65	1.9	2.3	2.7
Northwestern	− .05	1.0	1.4	2.25	3.0	4.35	5.65
Kansas	0.15	1.4	1.85	2.4	2.8	3.65	4.8
Northwestern	0.05	1.6	2.05	3.25	4.45	6.0	7.45

Chart 1 shows that polarization made at ¼ hour, ½ hour, and even at 1 hour intervals, under the condition of the Rumsey method, does not clearly show the relative saccharogenic activity of the flour diastase. Even 2 hours at 27° C. did not show distinctive differences between the flours. At the end of 4, or 7 hours' incubation at 27° C., consistent differences were revealed. While an incubation lasting 4–7 hours would be incon-

[1] *Loc. cit.*

venient and cumbersome this work indicates that it is desirable so to conduct the autolysis that substantial polarization increases are obtained; if the incubation periods are too short the susceptibility to attack of a small fraction of the starch of the flour is likely to obscure the effective saccharogenic power. Another reason for so conducting the tests that substantial polarization increases are obtained is that the polarimetric error of about $\pm 0.05°$ V. becomes relatively lower the higher the readings.
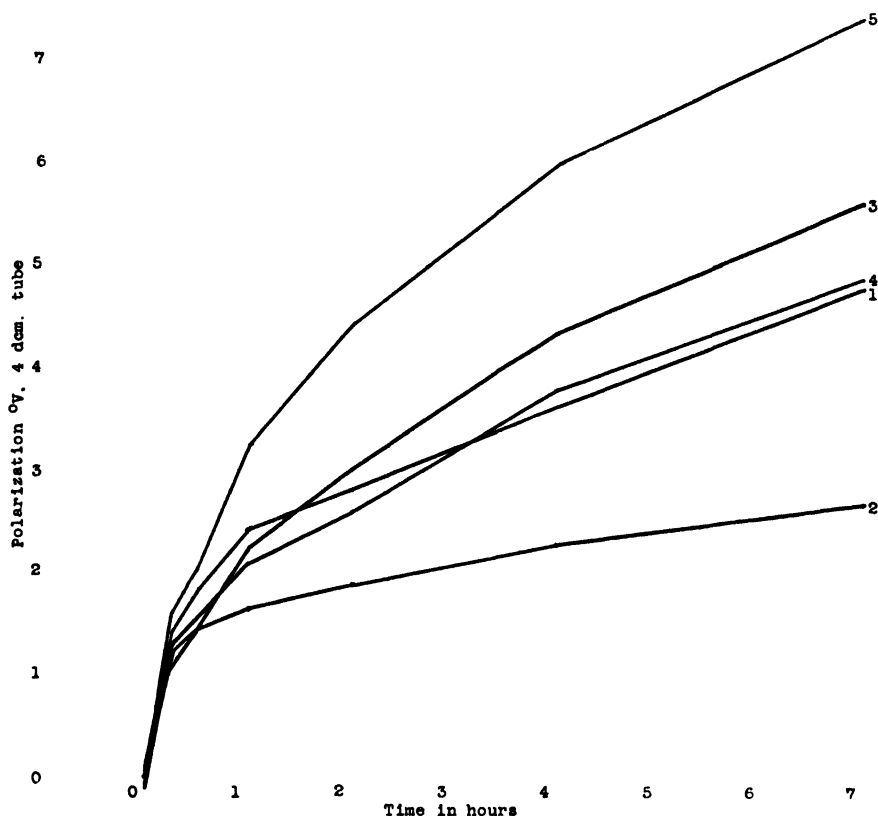
CHART 1. INCREASES IN POLARIZATION OF FILTRATES OF FIVE FLOUR SUSPENSIONS DURING AUTOLYSIS AT 27°C.

Since diastatic action can be increased by raising the temperature a series of tests was made in the manner above described except that incubation was conducted at 40° C. and 50° C., respectively, instead of digestion at 27° C. After the autolysis for 1 hour the samples were rapidly cooled in a cold water bath to room temperature before the sodium tungstate and sulfuric acid were added. All the polarizations were made within half an hour from the time the acid was added. The following data were obtained:

FLOUR	DIGESTION TEMPERATURE °C.	POLARIZATION °V. 4 DCM. TUBE
1. Kansas	40	—
	50	8.2
2. Texas	40	2.95
	50	4.65
3. Northwestern	40	4.7
	50	8.9
4. Kansas	40	4.2
	50	7.4
5. Northwestern	40	7.2
	50	12.6

These tests showed that heating at 40° or 50° C. for 1 hour probably advanced the saccharogenesis much farther than would be the case in the normal proofing of dough and that possibly a lower temperature should be selected.

C. N. Frey suggested to the writer that the pH be adjusted to within the range of that of the normal dough batch and that if practicable a phosphate mixture be used as the buffer. The temperature selected was 37° C. and Sorensen's phosphate buffer, consisting of 53.116 grams of potassium dihydrogen phosphate (KH_2PO_4) and 1.78 grams of disodium phosphate ($Na_2HPO_4 \cdot 12H_2O$) dissolved in water and diluted to 6 liters (pH 5.288) was used. It was found necessary to use this buffer solution instead of distilled water in making up the flour suspensions to be certain of obtaining a pH of 5.4 or less. The new polarimetric method was then tried on four flours. The copper reducing action of the flour filtrates was also determined in order to obtain the relationship between the polarization and the reducing substances formed. The details of this work are as follows:

METHOD

Twenty-five grams of flour was mixed with 241.5 cc. of Sorensen's phosphate mixture of 5.288 pH, at 37° C. and incubated for 1 hour at 37° C., with mixing every 15 minutes. The mixture was cooled rapidly in a bath of ice water to about 20° C. Then 7.5 cc. of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$, 15 grams per 100 cc.) was added and well mixed, and 1 cc. of sulfuric acid was added with continuous mixing. The flour suspension was then immediately filtered and the filtrate polarized, preferably not over half an hour being allowed to elapse between the time of adding the sulfuric acid and the polarization. The check determinations were made by mixing 25 grams of flour with a mixture of 241.5 cc. of water, 7.5 cc. of the sodium tungstate and 1 cc. of sulfuric acid.

The quantities of flour sample and solution given are such that sufficient

TABLE 1

Polarizations and increases in reducing substances in flour suspension filtrates. (Suspensions incubated for 1 hour at 37° C. at 5.4 pH.)

FLOUR	POLARIZATION			REDUCING SUBSTANCE INCREASES				
	INITIAL °V.	FINAL °V.	INCREASE °V.	INITIAL CuO	FINAL CuO	INC. CuO	MALTOSE % OF FLOUR	INCREASE PER 1°V. INCREASE IN POLARIZATION
				gram	gram	gram		percent
1. Oklahoma	0.5	8.0	7.5	0.010	0.270	0.260	3.67	0.49
2. Texas	−0.05	2.5	2.55	0.013	0.146	0.133	1.86	0.73
3. Northwestern	±0.0	4.3	4.3	0.009	0.172	0.163	2.28	0.53
4. Northwestern	+0.1	6.9	7.0	0.009	0.244	0.235	3.32	0.47

filtrate for polarization is obtained in half an hour or less. In using the Munson and Walker method it was necessary to dilute largely with freshly boiled hot water before filtering, as otherwise filtration was frequently excessively slow. This was especially true in case of the checks. Sufficient strong alkali was added to the Fehling's solution to neutralize the sulfuric acid present in the aliquot used, as suggested by Rumsey.[1]

The cuprous oxide was slightly impure, so it was converted to cupric oxide by ignition in a muffle as directed by Browne.[2] For example, the Cu_2O from 50 cc. of filtrate of flour No. 1. weighed 0.248 gram. The CuO weighed 0.270 gram instead of the computed weight of 0.276 gram.

The above polarimetric method is proposed for trial. There are many facts not well understood about saccharogenesis. This is illustrated by the fact that the increases in percentage reducing substance reckoned as maltose, for each 1° V. increase in polarization, are not constant but vary from one flour to another, as is clearly shown in Table 1.

EXPERIENCES WITH THE RUMSEY METHOD

In connection with the work leading to the formulation of the above polarimetric method, two faults were found with the Rumsey method. The cuprous oxide formed is somewhat impure, causing plus errors if the activity is computed from the weights of cuprous oxide. A more serious error is due to the gradual increase in reducing power which takes place in the flour suspension filtrate, doubtless caused by the presence of cane sugar, raffinose, tri-fructosan, or other fructoside. One or several of these substances apparently are slowly hydrolyzed in the presence of the sulfuric acid added to stop the autolysis, producing plus errors which increase in magnitude with the time which elapses after the sulfuric acid is added, until the solution is actually mixed with Fehling's solution. Curiously enough the increases in reducing power were greater in case of the filtrates of flour suspensions which had been incubated for 1 hour at 27° C. than in case of the filtrates of the non-incubated check samples. Some of the data obtained are given in Table 2.

All the checks increased in reducing power by values ranging from 4 to 14 mg. Obviously the time of standing even in case of the checks is a factor of importance. The polarizations of the checks did not change significantly. The incubated samples of acidified flour filtrate increased in activity by from 16 to 30 mg., and the polarization declined by from 0.05 to 0.25° V. In another experiment a flour filtrate of a Kansas Spring Wheat flour was prepared by rapidly filtering a flour suspension which had been incubated for one hour at 27° C., then treated with sodium tungstate and sulfuric acid, as in the Rumsey method. After the filtration, which required 1 hour, the filtrate was allowed to stand at 27° C. The reducing

power was measured on 50 cc. samples at intervals up to $3\frac{1}{2}$ hours by the Munson and Walker method. The data obtained are as follows:

TIME OF STANDING AFTER FILTRATION *hours*	Cu₂O FORMED *gram*	Cu₂O INCREASE *per cent*
0	0.091	—
$1\frac{1}{4}$	0.106	16
2	0.117	28
$3\frac{1}{4}$	0.130	43

A sample of the filtrate neutralized by dry sodium carbonate did not change in copper reducing power on standing for 4 hours at about 27°

TABLE 2

Increases in reducing power and decreases in polarization of flour suspension in filtrates on standing (Rumsey method).

SAMPLE	INTERVAL AFTER ADDITION OF Na₇WO₄ AND H₂SO₄	Cu₂O	INCREASE	POLARIZATION	CHANGE
	hours	*gram*	*gram*		
1. Kansas	$\frac{1}{2}$	0.014		−0.1	
	4	0.021	0.007	±0.0	
2. Texas	$\frac{1}{2}$	0.018		±0.0	
	4	0.022	0.004	±0.0	
3. Northwestern	$\frac{1}{2}$	0.015		−0.05	
	4	0.029	0.014	±0.0	
4. Kansas	$\frac{1}{2}$	0.017		−0.15	
	4	0.025	0 008	+0.2	
5. Northwestern	$\frac{1}{2}$	0.017		+0.05	
	4	0.026	0.009	+0.1	
Incubated Lots					
1. Kansas	$\frac{1}{2}$	0.103	0.016	2.1	−0.2
	4	0.119		1.9	
2. Texas	$\frac{1}{2}$	0.092		1.65	
	4	—	—	1.5	−0.15
3. Northwestern	$\frac{1}{2}$	0.111		2.25	
	4	0.125	0.014	2.2	−0.05
4. Kansas	$\frac{1}{2}$	0.102		2.4	
	4	0.132	0.030	2.25	−0.15
5. Northwestern	$\frac{1}{2}$	0.127		3.25	
	4	0.150	0.023	3.0	−0 25

C. This confirms Rumsey's findings,[1] namely that his filtered checks containing no added acid did not increase in reducing power on standing.

[1] *Loc. cit.*

The above data gives an idea of the magnitude of the errors due to letting the flour filtrates stand before analyzing them.

Berliner and Rüter[1] and also Blish[1] and co-workers found that marked increases in reducing power occurred upon treatment of the filtrate with hydrochloric acid as in the Clerget method in sugar analysis.

The writer has found that invertase also effects a marked increase in reducing sugars. This is shown by the experiment described below:

Two hundred grams of a soft wheat flour was mixed with 2000 cc. of water at 27° C. and incubated at the same temperature for an hour. Then the suspension was centrifugalized for a few minutes and the supernatant liquor filtered, a little kieselguhr being used as filter aid. Five hundred cc. of the clear filtrate was mixed with 5 cc. of an active solution of commercial invertase (K = 0.20), and the reducing power measured at intervals, 50 cc. samples being used. The sample was kept at room temperature preserved with toluene. The results follow:

INTERVAL	Cu_2O	Cu_2O- 5G. FLOUR	INCREASE DUE TO INVERTASE PER 5G. FLOUR—		SUGAR, AS INVERT FORMED, % OF
			AS Ca_2O	AS INVERT SUGAR	FLOUR
hours	*gram*	*gram*	*gram*	*gram*	
0	0.145	0.147	—	—	—
¼	0.211	0.213	0.066	0.030	0.59
½	0.220	0.222	0.075	0.034	0.67
1	0.234	0.236	0.089	0.040	0.80
2	0.240	0.243	0.096	0.043	0.86
20	0.270	0.273	0.126	0.057	1.14

The cuprous oxide was somewhat impure due to the fact that the Munson and Walker method was used on the straight flour filtrate containing organic impurities derived from the soluble protein of both the filtrate and the invertase. The results however show that the inversion was very rapid at first, then gradually slackened.

SUMMARY

The manifestations of diastatic activity in the dough batch are described,—namely, starch hydration, saccharogenesis, liquefaction, and saccharification—and the relative activities of the three last-named functions, to which it is possible to give numerical values, are discussed. The saccharogenic power is by far the slowest of the reactions and it is therefore believed to be the most important of the three types of diastatic action in flours.

The difficulties inherent in the problem of measuring saccharogenic activity due to the lack of uniformity of the "attackability" of the starch are described.

A polarimetric method for measuring diastatic activity in flour suspension is given; it is believed to be suitable for distinguishing between

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[1] *Loc. cit.*

SET SCREW

SWITCHES

WOOD BASE

24"

SET SCREW

23"

28"
STAND

½"x⅛"x3⅛"BRASS BAR

½"x½"x½"
½"x½"x1"IRON BAR

E

3⅛"

4½"

1½"

WOOD BASE

9"

END VIEW

¾"PIPE

SET SCREW

½" FLOOR FLANGE

9"

6"

WOOD BASE

STAND

8⅝"
13"

SWITCHES

½"x1"STRAP IRON

END VIEW OF "E"

30 GAUGE—50 OHMS

BRASS TUBE

LEVEL OF
PARACYMENE

2" BRASS
TUBE

80 10 0 BRASS TUBE

FIBRE RING
FIBRE BUSHING

SECTION THRU
TERMINALS "T"

SECTION THRU
RINGS "G"
(BRASS)

BRAZE

"2-56 RD HD SC.

"2-56 RD HD SC.

SLIDING
FIT

ADJUSTABLE SUPPORT
FOR TUBES
(BRASS)

CROSS SECTION THRU "E" SECTION THRU "E"

SECTION THRU "E"

HINGED COVER

"SIL-O-CEL"

30 DURICHEL
TUBE

"SUPEREX"

END VIEW OF "D"

SECTION THRU "D"

M

L

E

D

C

B

A

HEATING COILS OF CHROMEL "A" WIRE.
SECTION X-25 GA.—35 OHMS
SECTION B-28 GA.—25 OHMS
SECTION C-28 GA.—80 OHMS

AREA INSULATION
ASBESTOS TUBE

30 DURICHEL
TUBE

"SUPEREX" IN C
SIL-O-CEL IN A&B

TYPICAL SECTION THRU "A"&"C"   CROSS SECTION THRU "D"

NOTE: SHELLS TO BE 4" BRASS TUBE, ¼"WALL.
NOTE: ALL SCREWS TO BE "2-56 RD.HD.BRASS
MACH. SCREWS UNLESS OTHERWISE NOTED.

"2-56 MACH. SCREWS
WITH HEADS REMOVED

⅛"ASBESTOS BOARD

VIEW SHOWING METHOD
OF HOLDING TUBES IN PLACE

25 CM CuO Coil For "A"

12.5 CM   10 CM

14MM   12.5 CM

SECTION THRU "D"

63MM   CuO coil for "B"

75MM

14MM

60 CM   8   4CM

8   2.5
MM   1 CM

CaO₂ Ag Coil
Ag Coil CaO₂
MM

8   8CM   9CM
MM

115 CM

Ag Coil Ag Coil Asbestos
CuO, PbO₂
CaO₂

DIAGRAM SHOWING FILLING OF COMBUSTION TUBES

SW

H5-45 OHMS

H4-11 OHMS

H3-25 OHMS

H2-25 OHMS

H1-45 OHMS

CaCl₂

K

ASCARITE

SW

the saccharogenic activity of different flours and other dough batch ingredients.

In comparing the proposed polarimetric method with reducing sugar increases obtained by copper reduction, the quantitative relations between the two methods were found to vary from one flour to another for reasons not understood.

The Rumsey method was found defective in that the cuprous oxide was found to be not sufficiently pure for weighing directly. A more serious error consisted of the gradual increase in reducing power which took place in the flour suspension and its filtrate, probably due to hydrolysis of soluble fructosides by the sulfuric acid used in clarifying.

In conclusion the writer wishes to thank C. N. Frey, Director of The Fleischmann Laboratories, for his interest and for many suggestions.

SEMIMICRO DETERMINATION OF CARBON AND HYDROGEN

By E. P. Clark (Bureau of Chemistry and Soils,* U. S. Department of Agriculture, Washington, D. C.)

The following procedure for the determination of carbon and hydrogen in samples of the order of 20–25 mg. presents the details of one of the methods considered in the system of organic microanalysis outlined at the 1932 meeting of the A.O.A.C.[1]

As satisfactory combustion analyses upon samples of the size under consideration depend largely upon the use of appropriate apparatus, specifications for a furnace designed for this work are presented (Fig. 1) together with the following information.

The variable resistances, H1 to H5, inclusive, are Cenco air-cooled laboratory rheostats No. 4967. A 10-ohm variable resistance of the same type (not shown in the diagram) is inserted in the main power line leading to the furnace but not to the preheater A. The switches indicated by SW are General Electric No. 1299, single-pole miniature flush tumble type.

The electrical wiring is placed in grooves on the under side of the wood base, from which the necessary leads pass through appropriately placed openings to the rheostats and heaters above. The nickel tubes A, B, C, and E, are insulated with mica sheets split to such thickness that they can be wrapped around the tube without breaking. The mica is tied in place with thin cotton thread.

The heating coils for all the units except E are made in the following manner. A wire of the required resistance is closely wound and secured upon a 3/32″ metal rod, heated to redness, and plunged into water, a treatment that removes all resilience from chromel wire. The coil is then

removed from the rod by twisting it in the opposite direction from which it was wound, and the individual turns of the coil are then separated, but to compensate for end heat losses they are more closely spaced at the ends than in the central part of the units. For insulation purposes the coils are inserted in a tube of braided asbestos sleeving. The heating elements thus prepared are wrapped as closely as possible around the nickel cores of the units and the ends are secured to the lead wires.

The resistance wire in unit E is wound in the grooves of a threaded section of transit capillary tubing and returned to its outside connection through the capillary bore. Before the heating cartridge is placed in its brass receptacle it is insulated with thin sheet mica which is tied securely with No. 35 chromel wire. (Obviously other types of cartridge heating elements could be used advantageously in this unit.)

The end pieces attached to the ring G of the units A, B, C and D are made of asbestos board but the end pieces of section E are made of transit.

The Superex[1] insulation in the lid of D is held by two imbedded 3/4″ washers attached to the lid by machine screws. The exposed Superex is painted with a thin aqueous paste of Rutland furnace cement to give it a clean hard surface.

The paracymene jacket (section E) is soldered with lead and joined to the glass air condenser with a cork stopper. The cork connections are made vapor tight with Rutland furnace cement.

Tube Filling and Reagents:

The exclusive use of oxygen for the entire combustion has been most satisfactory for all substances encountered. Tank oxygen enters the system through the bubble counter J at the rate of 5 cc. per minute. It is purified by passing through the hot copper oxide wire in the preheater A and the U tube K.

The reagents for the combustion tube except the pumice supported cerium dioxide catalyst are prepared according to the directions of Pregl.[2] The latter is made as follows: Three grams of 12-mesh pumice stone is thoroughly impregnated with a solution of 5 grams of pure cerium nitrate dissolved in 12 cc. of water. The mixture, contained in a porcelain dish, is dried on a steam bath with frequent stirring to prevent caking. It is then transferred to a combustion tube, which is placed in a furnace and slowly heated to ca. 550° in a current of oxygen. Under this treatment the cerium nitrate is converted to cerium dioxide.

Scotch Moncrieff combustion tubing[3] is used because of its excellent qualities and its availability in the desired size.

[1] A. Johns Manville Company high temperature insulator which is marketed in blocks of various sizes.
[2] F. Pregl. Quantitative Organic Microanalysis. Translated by Fyleman. Blakiston's Sons and Co. Philadelphia, 1930. Page 30.
[3] Obtained from Jenkins Bros., Philadelphia, Pa.

Absorption Train:

The glass-stoppered absorption tubes L and M are 14 by 100 mm. The end connecting arms are made of capillary tubing 4 mm. outside diameter with a 1 mm. bore. L and M contained respectively 12-mesh calcium chloride and ascarite. The reagents are held in place by small plugs of cotton at the ends of the tubes. To prevent early blocking of the ascarite tube, thus assuring its use for eight to ten combustions, two sections of 1 mm. melting point tubing are inserted in the fore end of the absorbent. One tube, ca. 13 mm. long, is placed in the reagent to a depth of 10 mm.; the other tube, ca. 23 mm. long, is embedded to a depth of 20 mm. These tubes are readily dropped in place as the absorption tube is filled. The ground stoppers are then sealed with Krönig's glass cement (1 part white wax and 4 parts rosin).

A third tube attached to M one half of which contains ascarite and the other half calcium chloride is also used as a guard tube but is never weighed. The rubber connections of the tubes of the absorption train and also the bore of the stopper connecting the train to the combustion tube are lubricated with glycerine as directed by Pregl.[1]

When the entire equipment, with the exception of the absorption train, is assembled, the units A, B, C and D are heated to ca. 550° (a dull red heat), and oxygen is passed through the system for 2 hours. Sufficient heat is also applied to the paracymene bath to keep the vapors of the hydrocarbon constantly refluxing from the lower bulb of the condenser. (Attention is directed to the mistake so frequently made of heating the combustion tube to excessively high temperatures. A bright red heat is not only unnecessary but undesirable as the glass may soften, become distorted or even blow out and also fuse to the reagents, which will cause the tube upon cooling to crack. A combustion tube used by the writer as directed has been employed for more than 300 determinations, yet it is apparently as good as new.)

After this preliminary treatment the apparatus is ready for use.

The Combustion:

A combustion procedure which has given uniformly good results upon a large variety of compounds is as follows.

Heat is applied to the preheater A and section D and E, while oxygen at the rate of ca. 5 cc. a minute is admitted to the apparatus through J. As soon as the temperature of the units is approximately 550° and the paracymene is refluxing, the copper oxide coil in B is removed, and 10 to 15 mg. of a pure substance, such as sugar, contained in a platinum boat 35 mm. long, 8 mm. wide at the top, 5 mm. wide at the bottom and 6 mm. deep is placed in the tube between sections B and C. The copper oxide coil is replaced, the tube is connected with the oxygen supply, the absorp-

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[1] *Loc. cit.*, page 52.

tion train with the extra guard tube is connected to the combustion tube, and finally heat is applied to both B and C. Usually the temperature of C, the burning unit, is a little higher than that of the other units. C is then caused to approach the boat at such a rate that a slow steady combustion occurs. Usually about 15 minutes is required to burn the sugar sample. A small flask containing palladious chloride solutions is connected to the end guard tube by means of thin-walled, small-bored rubber tubing. The reagent is made by diluting 1 cc. of a 5 per cent solution with 200 cc. of water. The unused oxygen from the absorption train bubbles through this solution, which functions first as a carbon monoxide detector. A positive test, which would invalidate a determination, rarely occurs, but if it should, it would indicate that the combustion was too rapid or that the supply of oxygen was insufficient. The flow of gas through the palladious chloride solution as compared with that passing through J also gives an indication of the rate of absorption of oxygen. The quantity of oxygen admitted to the apparatus and the rate at which the sample is burned should always be regulated so that an excess of the gas bubbles through the palladious chloride solution. After the sugar is completely burned, the heat in sections B and C is turned off, and the combustion tube is swept out for approximately 20 minutes by continuing the regular flow of oxygen. During the combustion and sweeping out of the tube, a gentle flame is occasionally applied to the end of the combustion tube to which the absorption train is connected to prevent moisture from condensing at this point.

The absorption train is next disconnected from the combustion tube, the rubber stopper on the calcium chloride tube is replaced by a rubber nipple, and the train is allowed to adjust itself to the temperature of the balance for a period of 5 minutes. The tubes are then disconnected, the capillary end arms are stoppered, and the ascarite and calcium chloride tubes are weighed to at least 0.1 mg. in the order named. During the weighing the rubber nipples are removed, and another sealed tube of the same size but slightly lighter than these being weighed is used as a tare on the right balance hook. Any difference due to atmospheric changes, buoyancy of air and other factors is in this way eliminated. As the practice of wiping the tubes before weighing showed no advantage, it was eliminated. It is advisable to hold the tubes by the capillary ends during all necessary manipulations.

After being weighed the tubes are again assembled and connected to the combustion tube. A 20–25 mg. sample of the material to be analyzed, which has previously been weighed in a platinum boat to 0.01 mg., is placed in the tube and burned as directed for the sugar sample. The time required for its combustion is usually 20 minutes, but this varies somewhat depending upon the nature of the substance. After the system has been swept out and the tubes allowed to cool the latter are weighed in

the same order as before. The increase in weight of the calcium chloride and ascarite tubes is, of course, due to the water and carbon dioxide formed during the combustion.

The preliminary burning of a small sample of sugar or other material is to establish an equilibrium in the entire system comparable to that which exists during an actual determination. This is especially important when the lead peroxide unit is employed, because this reagent is particularly sensitive to environmental changes.

When the work warrants, it is suggested that two combustion tubes be available, one having lead peroxide for use in burning nitrogenous compounds and another without the lead peroxide for use in burning nitrogen-free compounds. When the latter is used the paracymene unit is, of course, removed. These tubes may be readily interchanged, and when a considerable number of nitrogen-free compounds are to be analyzed, time is saved in sweeping out the apparatus.

The results obtained with the apparatus and the procedure described have been uniformly of a degree of accuracy expected from the precision with which the samples and combustion products were weighed.

A few typical results are presented in the following table.

Typical carbon and hydrogen analyses of samples of the order of 20–25 mg.

SUBSTANCE	CALCULATED		FOUND	
	C	H	C	H
	per cent		per cent	
Dihydrotoxicarol $C_{23}H_{24}O_7$	67.08	5.89	66.97	5.87
Dihydrodesoxytoxicarol $C_{23}H_{26}O_6$	69.32	6.58	69.29	6.66
2-Hydroxy-4, 5-dimethoxy benzoic acid, $C_9H_{10}O_5$	54.54	5.09	54.46	5.20
Deguelin $C_{23}H_{22}O_6$	70.03	5.63	69.99	5.63
Tephrosin $C_{23}H_{22}O_7$	67.30	5.41	67.31	5.47
Apotoxicarol $C_{18}H_{16}O_7$	62.78	4.69	62.91	4.81
Gossypol dianilide $C_{42}H_{40}N_2O_6$	75.43	6.03	75.35	6.12

AN ELECTRICALLY HEATED SAND BATH*

By E. P. CLARK (Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.)

An electrically heated sand bath has a wide range of utility in the chemical laboratory, and for many purposes it is superior to other methods of heating.

Specifications for a useful form of such an apparatus are given in the accompanying drawing. It consists of a transite box assembled with machine screws and supported near its four bottom corners by 1/2 inch metal studs. It is lined as shown with 1-1/4 inch superex insulation,

* Contribution from the Insecticide Division.

equipped with a heating element placed in the bottom of the box,[1] and filled to within 1/2 inch of the top with clean white sand.

During operation the temperature of the surface sand increases from the sides to the center of the box, and the temperature within the sand increases with its depth. With these characteristics, the apparatus affords means of evaporating many liquids at temperatures at or below their boiling points. For this purpose the container is adjusted at the proper place on the sand surface or if necessary imbedded sufficiently deep in the loose
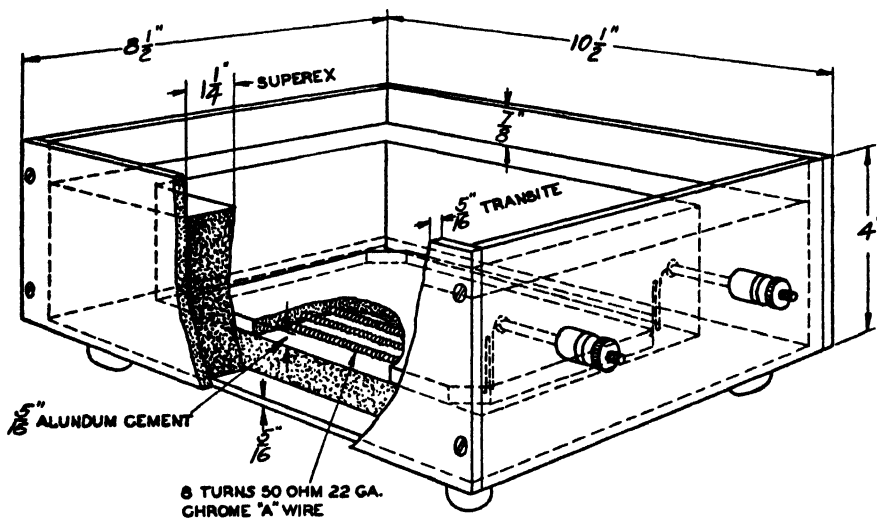
ELECTRICALLY HEATED SAND BATH

sand. The apparatus is especially useful for refluxing liquids, as air condensers may safely be used even for such low-boiling materials as acetone. It is also useful for quickly heating liquids and mixtures in test tube experiments. The test tube, with its contents, is plunged into the loose sand and heated to the desired temperature in a few seconds.

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[1] The heating element may be easily constructed as follows: The resistance wire is closely wound and secured upon a piece of metal rod of such gage as to give the coil the desired outside diameter. Resilience is removed by heating the wire to redness and plunging it into water. The coil is then removed from the rod and uniformly stretched to the proper length. It may then be placed upon a stiff sheet of mica or, preferably, imbedded in an alundum plate as follows: The coil, held in an appropriate frame, is covered with a thick uniform aqueous paste of RA305 alundum cement. The form is dried at 80–90°C., removed from its frame to a tile, and heated to redness in a muffle furnace. This transposes the cement to a hard plate in which the coil is imbedded.

In the selection of this, or other forms of the apparatus which may suggest themselves, the following advantages should be considered: The cost and current consumption are low; the working range is wide and may be easily varied; and when inflammable liquids (except carbon bisulfide) are being used no fire hazards are encountered.

IDENTIFICATION OF FLAVORING CONSTITUENTS OF COMMERCIAL FLAVORS

III. Identification of Gamma-undecalactone

By JOHN B. WILSON and GEORGE L. KEENAN[1]

Gamma-undecalactone has been used for a decade or more as an ingredient of imitation peach and apricot flavors, and heretofore it has been identified in such products by its characteristic odor and flavor alone. This substance, known to the trade under such varying names as "Peach Aldehyde," "C-14 Aldehyde," "Peche,"[2] "C-16 Aldehyde,"[2] was widely used in perfumery for a number of years before its more recent adoption as an ingredient of imitation flavors and as an adulterant of the so-called true-fruit flavors.

Chemically γ-undecalactone is the cyclic ester resulting from the dehydration of γ-hydroxyundecyclic acid, which occurs at ordinary temperatures when salts of the acid are decomposed by the addition of a stronger acid to their water solution according to the following equation:

$$CH_3(CH_2)_6CH(OH)CH_2CH_2COONa + HCl = CH_3(CH_2)_6$$
$$CHCH_2CH_2CO + NaCl + H_2O$$
$$\underline{\qquad O \qquad}$$
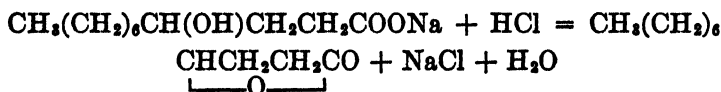
The lactone separates out of the solution in the form of an oily liquid. In concentrated form the odor of this liquid is similar to that of castor oil or coconut oil, but in dilute solution it bears a remarkable resemblance to that of peaches.

Shukow and Schestakow[3] prepared γ-undecalactone from undecylenic acid by heating with concentrated sulfuric acid. Durrans[2] indicates that this method is used commercially, the undecylenic acid being obtained from the destructive distillation of castor oil. Following the procedure of Shukow and Schestakow, the writers prepared a small quantity of the lactone in the laboratory for experimental work.

A quantity of γ-undecalactone was dissolved by heating with sodium hydroxide solution. The excess alkali was neutralized with sulfuric acid. Two portions of the solution containing sodium salts equivalent to about 200 mg. of the lactone were diluted to 50 cc. with water and 2 cc. of a

[1] Joint contribution from the Water and Beverage Section of Food Control, and Microanalytical Laboratory, Food and Drug Administration, U. S. Department of Agriculture.
[2] Perfumery and Essential Oil Record Year Book and Diary for 1929.
[3] *J. Russ. Phys. Chem. Soc.*, **40**, 830 (1908).

neutral 20 per cent solution of silver nitrate was added to each. A heavy precipitate formed at once. The mixtures were stirred vigorously for a few moments and set aside in a dark place for 30 minutes. The precipitates were then filtered, washed thoroughly with water, and dried in a vacuum oven at 70°C. Silver was determined in the precipitates by ashing.

WEIGHT TAKEN	Ag FOUND	
gram	*gram*	*per cent*
0.240	0.0836	34.83
0.105	0.0367	34.95

Calculated for $Ag(C_{11}H_{21}O_3)$ 34.91

Some of the lactone was treated with hydrazine hydrate. Hydrazino-γ-undecalactone formed, as described by Blaise and Luttringer.[1] After this substance had been recrystallized from butyl alcohol it was examined under the microscope, and the optical properties were determined by the method described in Part I[2] of this series. The substance was found to contain 13.03 per cent of nitrogen by the Dumas method (N = 12.95% in $C_{11}H_{24}O_2N_2$.).

Optical properties of hydrazino-γ-undecalactone.—In ordinary light the substance is seen to consist of lath-like rods, many of them more or less split at the ends. In parallel polarized light (crossed nicols), the substance is characterized by not extinguishing sharply, most of the rods remaining essentially bright when the stage is rotated. Occasionally there are found crystals that extinguish sharply, have square ends, and show straight extinction and negative elongation. In convergent polarized light (crossed nicols) partial biaxial interference figures, usually showing one optic axis up or slightly inclined to the normal, are of frequent occurrence. The refractive indices, as determined by the immersion method, are as follows: $\alpha = 1.483$ (not common); $\beta = 1.525$ (most frequently occurring of the indices and shown lengthwise on rods); $\gamma = 1.555$ (occurring crosswise on rods which show straight extinction and negative elongation); all ± 0.003.

After a number of trials it was found that the procedure presented could be depended upon to yield crystals of hydrazino-γ-undecalactone which could easily be identified under the microscope by the determinative data given above. The test follows:

QUALITATIVE TEST

To fractions A and G obtained by carrying out the procedure for the detection and qualitative separation of classes,[3] add a few drops of hydrazine hydrate solution (42 per cent in water) and mix thoroughly; if γ-undecalactone is present in sufficient quantity a white solid will begin to separate in a short time. Allow the mixture to stand 15–20 minutes. (If the fraction is composed largely of γ-undecalactone, the material will be nearly solid and an ammoniacal odor will be detected.) Place the mixture on the steam bath and heat until the ammoniacal odor is no longer evident.

[1] *Compt. rend.*, **140**, 790 (1905).
[2] *This Journal*, **13**, 389 (1930).
[3] *Ibid.*, **15**, 639 (1932).

Add about 1 cc. of normal butyl alcohol and warm until a clear solution is obtained, adding a few additional drops of the alcohol, if necessary, to dissolve the residue completely. Remove the beaker from the steam bath and permit the butyl alcohol to evaporate spontaneously. (This usually occurs overnight, but a longer time may be necessary if much butyl alcohol has been used.) Examine the colorless or slightly yellowish crystals under the microscope. Hydrazino- γ-undecalactone has a characteristic odor similar to that of the lactone itself.

Examination of commercial samples.—Four commercial products labeled "Peach Aldehyde," one labeled "C-14 Aldehyde," and one labeled "C-14 Aldehyde 100%" were found to respond to the test giving crystals easily identified under the microscope as hydrazino-γ-undecalactone. Two other commercial samples labeled "Aldehyde C-14" and one labeled "Pseudo Aldehyde C-14" did not give crystalline material when the test was applied, showing that they contained no γ-undecalactone but were substances of an entirely different composition. These products were also lacking in the characteristic odor associated with this lactone.

Examination of laboratory samples.—An imitation peach flavor containing in 50 cc., 0.55 gram of commercial "Peach Aldehyde" and in addition known quantities of sucrose, citric acid, sodium benzoate, vanillin, benzaldehyde, capryllic acid, amyl butyrate, alcohol and water, was carried through the procedure for qualitative separation of classes,[1] and several fractions were tested for γ-undecalactone by the procedure given above. Gamma-undecalactone was found in fraction G, but in no other fraction. The test was negative when applied to fraction A, which consisted of a small quantity of yellowish oily material, showing that the γ-undecalactone had been completely volatilized by steam. However, it seems advisable to make the test on fraction A as a precautionary measure, as the lactone might not be completely volatilized by steam in the distillation and if it were present in the form of an alkali salt of γ-hydroxyundecylic acid it would be removed from the sample only after acidifying with sulfuric acid, by the extraction with ether.

A similar separation and test was made upon 500 cc. of sirup containing 20 cc. of the same flavor used previously and 0.6 gram of additional sodium benzoate. In this case (in which 0.22 gram was present in the sirup) the test for γ-undecalactone was positive when applied to fraction G and negative on fraction A.

SUMMARY

Hydrazino-γ-undecalactone was prepared, and its optical properties were ascertained by the immersion method.

A qualitative test is given for the identification of γ-undecalactone.

The test was applied with success to commercial preparations of this substance and to fractions obtained by the procedure for qualitative separation of classes in flavors and sirups given in Part II of this series.[1]

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[1] *This Journal*, 15, 639 (1932).

DETERMINATION OF SELENIUM IN WHEAT AND SOILS[1]

By W. O. ROBINSON (Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.)

Selenium can be separated from all the elements except arsenic and germanium by distillation of the chlorides or bromides (preferably the latter) from solutions of the respective acids. The distillate may be evaporated without loss of selenium by keeping it oxidized with addition of bromine water. Selenium may then be precipitated by the addition of hydroxylamine hydrochloride in an approximately 12 per cent hydrobromic acid solution. Elemental selenium appears as an orchid pink precipitate which can be filtered on an asbestos pad, dried, and weighed. Details of this procedure by which the writer was able to show the presence of very small quantities of selenium in wheat follow.

One hundred grams of wheat flour in a quartz dish was drenched with an aqueous solution of 10 grams of calcium acetate. The mixture was stirred thoroughly, dried on a steam bath or in a drying oven, and slowly ignited in an electric muffle furnace having a maximum temperature of 525° C. The dish was removed from the furnace twice, and the charred mass was turned and broken up with a spatula. When the lumps were ashed to an apparent gray, but the inside was still charred, the dish was removed from the furnace, cooled, and then drenched with water. The contents were taken up with 25 ml. of strong hydrobromic acid and 5 ml. of saturated bromine water. The acid solution was washed into a small distilling flask and distilled. The distillate was received into a small Erlenmeyer flask, surrounded by cracked ice, and containing 5 ml. of bromine water. When the distillation flask was nearly dry, the receiving flask, still surrounded by cracked ice, was disconnected, and a slow current of sulfur dioxide was bubbled through. When the bromine water was discolored, the tube was withdrawn, ¼ gram of hydroxylamine hydrochloride was added, and the flask was lightly stoppered and put on the steam bath for 2 hours. In most cases a characteristic orchid pink precipitate developed in a few minutes, but if no precipitate appeared, the flask was set aside for several days.

It was later found that in the case of wheat the preliminary distillation was unnecessary. The ash was treated with hydrobromic acid and bromine water and filtered. The filtrate was then treated as described for the distillate.

By this method duplicates of 10 and 12 parts per million of selenium (Se) were obtained in one subsample of toxic wheat, and 5 and 6 parts per million were obtained in another subsample of the same general lot of unmixed wheat. The gluten of the toxic wheat contained 90 parts per million of selenium. Samples of other non-toxic wheat yielded no selenium under the same treatment. When selenium was added to non-toxic wheat before ashing, nearly all could be recovered by the method given.

Oxidation of the wheat flour by hydrogen peroxide and by nitric and perchloric acids was unsatisfactory. Apparently the selenium was carried

over by the discharged gases. Oxidation with sodium peroxide was also unsatisfactory owing to the large amounts of reagents necessary (for 100 grams of wheat flour, 500 grams of sodium carbonate and 500 grams of sodium peroxide are required).

Selenium was found to the extent of 0.3 parts per million in a certain soil. A 100 gram sample was treated with aqua regia on the water bath, and filtered. A little sulfur dioxide, then hydrogen sulfide, was passed through the liquid. The precipitate containing sulfur, selenium, and so forth, was filtered, removed from the filter paper, and transferred to a closed glass tube. The bulk of the sulfur was cautiously volatilized, and the residue was dissolved in bromine water and hydrochloric acid. From this solution the selenium was precipitated in the usual manner.

RAPID COLORIMETRIC DETERMINATION OF TOTAL CARBON AND NITROGEN IN THE SAME SAMPLE[1]

By E. M. EMMERT (Agricultural Experiment Station, Lexington, Ky.)

A rapid determination of total nitrogen by conversion into nitrate and estimation colorimetrically was published several years ago.[2] More recently a rapid colorimetric method for the determination of carbon dioxide was published.[3] When these two methods were combined and some special apparatus for catching and measuring the gas evolved was used, it was found that total carbon and nitrogen could be determined rapidly and accurately in the same sample. The method follows:

Oxidation of sample and collection of the nitric acid and carbon dioxide.

Place the sample in the bottom of a 500 cc. Kjeldahl flask, taking care not to leave part of the sample in the neck or on the sides of the flask. Determine the size of the sample by the quantity of carbon and nitrogen present and the quantity of sodium chlorate required for the oxidation. (The sample should contain at least 0.5 mg. of nitrogen and 10 mg. of carbon and should not be so large that it takes more than 5 grams of sodium chlorate to oxidize it. This is determined by experimentation for each type of material. A 2 liter flask will hold the gases in ordinary determinations when 1–5 grams of sodium chlorate is used. The sample should be in a fairly fine state of division, especially if difficultly oxidizable.) Add the chlorate (1–2 grams will oxidize most samples) and place the flask in the apparatus as shown in Diagram 1. See that all connections are air tight and that stopcocks B and D are open. The 200 cc. flask (A) should contain 100 cc. of water, and C, 2 liters of 1 per cent by volume sulfuric acid. When all is ready add 25 cc. of 50 per cent by volume sulfuric acid to the sample in the Kjeldahl flask. If carbonate carbon is present, take special care to add the acid and make the connection to the condenser before any CO_2 can escape.

Heat the sample rapidly at first with a high flame. (This heats the air above the sample before the reactions start to any extent and decomposes any ClO_2 which
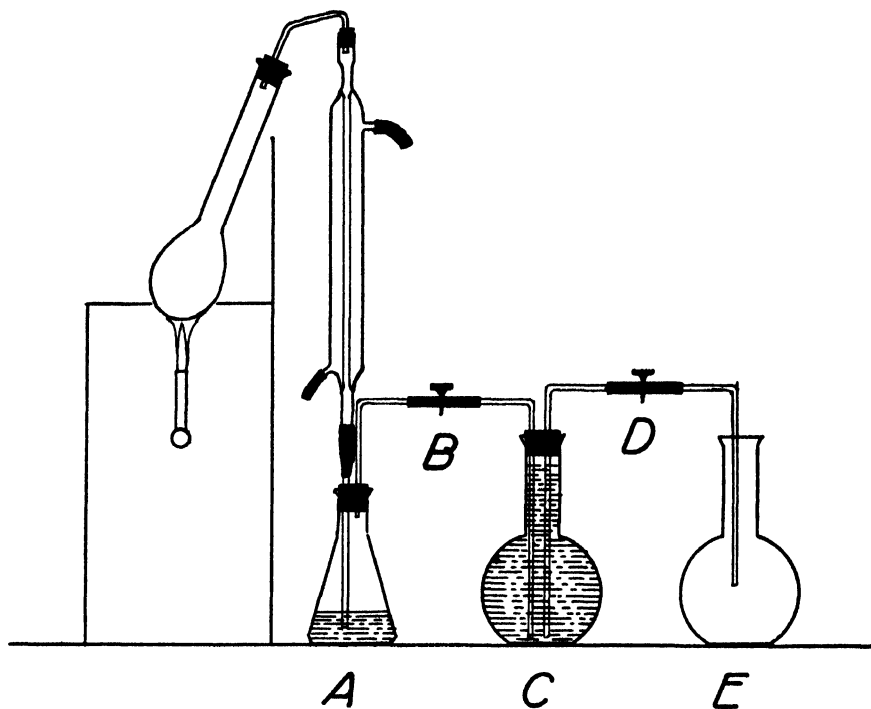
DIAGRAM 1
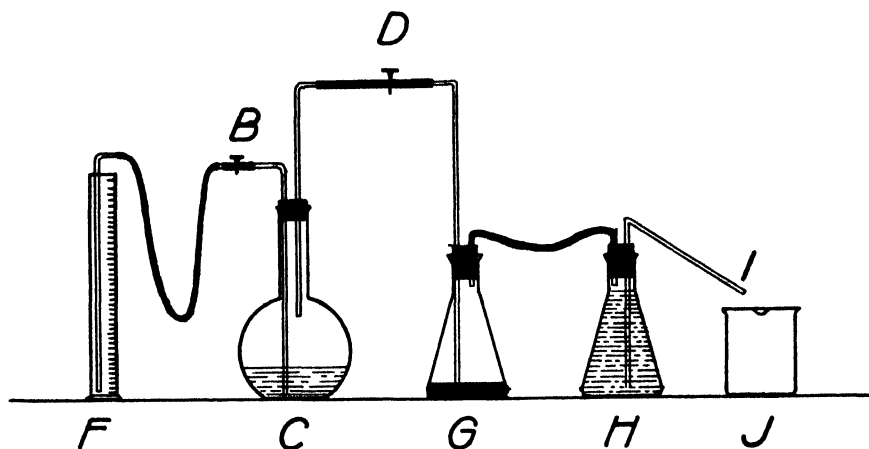
DIAGRAM 2

may form.) Lower the flame when the gases begin to form rapidly, and even remove altogether if necessary, but be ready to return it as soon as rapid evolution ceases. Continue heating until the reaction is complete, the water is distilled over, and clear

sulfuric acid remains and begins refluxing on the sides of the Kjeldahl flask. (The oxidation and distillation usually require about 10–15 minutes.) Disconnect the Kjeldahl from the condenser before turning off the heat and wash the condenser into A until it is filled to the stopper. Close stopcocks B and D. The nitrogen is present in A as nitric acid and the carbon in C as CO_2.

Detach C from A and E, leaving the stopcocks on C. Apply suction at D until a small vacuum is created in C and close D. Attach D to a glass tube which extends into a 20 per cent KI solution. Open D and allow about 10 cc. of KI solution for each gram of chlorate used to be drawn into C. Close D and shake C intermittently, but violently, for about 5 minutes. Iodine is liberated and the chlorine extracted from the CO_2 and air in C. The original volume of gas in C is restored by opening one of the stopcocks and allowing air to enter until no more is drawn in. Place the contents of A in a 500 cc. Erlenmeyer flask and boil rapidly while the CO_2 is being determined. Measure exactly the dilute sulfuric solution which was forced into E, which is the total volume of the gas forced from the Kjeldahl. After the chlorine has been extracted from C place C in the apparatus shown in Diagram 2.

Determination of Carbon Dioxide

Keep the tube to D above the solution in C. Put some water into G and fill the graduated cylinder F and flask H with water. Open B and D and start a siphon by applying suction at I. Close D. Remove the water from G and put an appropriate quantity of red sodium phenolphthalate solution in G.[1] Lower the level of water in F to that in C and open D, thus equalizing the pressure. Take the exact reading in F with the end of the tube from C at the water level and then raise F. (Gas will be forced from C into G and H.) Allow about 70–80 cc. to be forced over. (Water will flow from F into C and from H into J.) Again take the reading of the level in F when it is the same as that in C and also of the end of the tube from C. (The difference in reading will give the volume of gas forced into G.) Close D and shake G intermittently, but violently, until no further reduction of color is noted (about 5–10 minutes). Use sufficient rubber tubing to G to allow the shaking without interferring with C or H. Compare the reduced color with the original color of the phenolphthalate and compute the CO_2 which was introduced into G.[1] Divide this result by the cc. of gas forced into G. This gives the mg. of CO_2 per cc. in C. Repeat the operation of forcing gas from C into G and the determinations of CO_2 in the aliquots until the mg. of CO_2 per cc. of gas in C check satisfactorily. (Sometimes the values do not check on the first two aliquots due to faulty technic.) When it is certain the mg. of CO_2 per cc. of gas in C is correct, multiply by the total volume of gas as found previously and calculate the carbon in the sample. It is important to standardize the phenolphthalate solutions by titration against standard acid as their normality changes somewhat on standing.

DETERMINATION OF THE NITRIC ACID

While the carbon dioxide is being determined the solution in A containing the nitrogen as nitric acid should be boiled vigorously to expel chlorine, but the volume of solution should not be reduced below 50 cc. As the solution contains considerable chloride sufficient silver sulfate should be added and brought into solution to precipitate all the chloride. The nitric acid is determined as previously described.[2] Calculate the percentage of nitrogen in the sample from the nitrate nitrogen found.

[1] *This Journal,* 14, 386 (1931).
[2] *Ibid.,* 13, 146 (1930).

If interference in the yellow nitrate color occurs from some compound other than chloride acting on the phenoldisulfonic acid, use NaOH in excess to neutralize the phenoldisulfonic acid, make the yellow solution to volume, add about a gram of calcium hydroxide and shake with the solution a few minutes, and filter the solution. The foreign color usually disappears and a good color comparison may be made.

RESULTS

The results of carbon and nitrogen determinations on pure picric and sulfanilic acids are shown in the table. These compounds were chosen because they can be obtained pure, are stable, and not deliquescent. They are about as difficult to oxidize as most organic compounds, and contain both nitrogen and carbon.

Carbon and nitrogen in pure picric and sulfanilic acids.

ACID ADDED	CARBON ADDED THEORETICAL	CARBON FOUND	ERROR FROM THEORETICAL	NITROGEN ADDED THEORETICAL	NITROGEN FOUND	% ERROR FROM THEORETICAL
mg.	*mg*	*mg.*	*per cent*	*mg.*	*mg.*	*per cent*
			Picric Acid:			
100	31.412	31.800	+1.23	18.337	18.06	−1.74
100	31.412	31.447	+0.11	18.337	18.48	+0.77
50	15.706	15.770	+0.42	9.168	8.65	−5.64
50	15.706	15.824	+0.75	9.168	9.20	+0.34
50	15.706	15.561	−0.92	—	—	—
			Sulfanilic Acid:			
100	34.412	34.078	−0.96	6.692	6.67	−0.33
100	34.412	33.914	−1.32	6.692	6.54	−2.27

APPROXIMATION OF THE MILK SOLIDS OF A MILK PRODUCT BY MEANS OF ITS CITRIC ACID CONTENT

By B. G. HARTMANN and F. HILLIG (Food Control,[1] Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

In a previous issue of *This Journal*[2] the writers described a method for the determination of the citric acid content of milk.

Citric acid is present in milk as the calcium salt. According to Sherwood and Hammer[3] "335 citric acid determinations on milk from individual animals of the four principal dairy breeds varied from 0.07 to 0.33 per cent and averaged 0.18 per cent." For these determinations the Beau modification[4] of the method of Denige[5] was used. Sherwood and Hammer

[1] W. B. White, Chief.
[2] *15*, 643 (1932).
[3] Research Bull. No. 90, Iowa College (1926).
[4] *Rev. Gén. Lait,* 3, 385 (1904).
[5] *Ann. Chim. Phys.*, 18, 382 (1899).

found that the breed of animal has no significant effect on the citric acid content of the milk.

Contrary to the insistent claims in the literature that the pentabrom-acetone method for the determination of citric acid in milk is not reliable, the writers find not only that the method is accurate but that it is extremely simple in its operation and furnishes closely agreeing duplicates.

A collaborative study of the milk supply of 14 large cities located in various sections of this country show that the results obtained by the pentabromacetone method are fairly uniform. Fourteen analysts participated in the work. The 58 samples examined consisted of bottled milks taken from wagon deliveries. Generally speaking, the duplicates reported by the analysts checked closely, 70 per cent being within 3 mg. The duplicates of 9 and 18 mg., which were reported by one analyst, are due, it is believed, not to any fault in the method but rather to inexperience on the part of the analyst. The following tabulation presents the results obtained in the study:

TABLE 1

Citric acid content of bottled milks.

STATION	NUMBER OF OF SAMPLES	CITRIC ACID (ANHYDROUS)	
		per cent	*average per cent*
Washington	5	0.17, 0.16, 0.17, 0.15, 0.17	0.16
Baltimore	5	0.15, 0.15, 0.15, 0.15, 0.15	0.15
Boston	3	0.15, 0.17, 0.19	0.17
Buffalo	5	0.16, 0.17, 0.16, 0.16, 0.16	0.16
New Orleans	2	0.18, 0.17	0.18
Savannah	5	0.15, 0.14, 0.16, 0.17, 0.18	0.16
Cincinnati	4	0.17, 0.17, 0.17, 0.16	0.17
Minneapolis	2	0.15, 0.15	0.15
Chicago	6	0.15, 0.15, 0.16, 0.15, 0.17, 0.16	0.16
St. Louis	2	0.17, 0.17	0.17
Kansas City	6	0.14, 0.16, 0.16, 0.16, 0.17, 0.16	0.16
Denver	1	0.16	0.16
San Francisco	4	0.15, 0.14, 0.14, 0.14	0.14
Seattle	8	0.16, 0.16, 0.16, 0.15, 0.15, 0.16, 0.16, 0.16	0.16

Summary

Samples	5	16	20	14	2	1
Citric acid (per cent)	0.14	0.15	0.16	0.17	0.18	0.19

Average percentage of citric acid, 58 samples = 0.16; 50 samples = 0.16

The citric acid content of the bottled milks examined is practically uniform, 0.16 grams per 100 grams of milk. Therefore, as bottled milks

represent the composite of large herds, it seems reasonable to assume that the bulk milks used in the manufacture of milk products will contain 0.16 citric acid on the average and, based upon this assumption, a citric acid determination of an evaporated or sweetened condensed milk furnishes the means for calculating the content of milk solids. Furthermore, the citric acid content of a milk product furnishes the basis for calculating the milk solids of the original milk used in the production of a whole milk or skim milk powder.

The data presented in the literature relative to the solids content of fluid whole milk vary widely. Van Slyke reports an average of 12.9 per cent for 5,552 samples. According to a New York State report[1] the milk of 208 herds averaged 12.1 per cent solids and 3.65 per cent fat.

In Table 2 the calculated solids of the original milks used in the production of milk products are presented:

TABLE 2

Calculated solids of milks used in production of commercial milk products.

MILK PRODUCT	SOLIDS	CITRIC ACID	ORIGINAL MILK	
			SOLIDS	CITRIC ACID
	per cent	*per cent*	*per cent*	*per cent*
Whole Milk Powder	98.3	1.34	11.7	0.16
Whole Milk Powder	97.6	1.26	12.4	0.16
Whole Milk Powder	96.4	1.26	12.2	0.16
Whole Milk Powder	95.2	1.32	11.5	0.17
Skim Milk Powder	95.2	1.84	11.8	0.17
Skim Milk Powder	96.4	1.78	12.2	0.16
Skim Milk Powder	97.0	1.85	12.0	0.16
Skim Milk Powder	96.7	1.79	12.2	0.16
Skim Milk Powder	96.8	1.81	12.1	0.16
Evaporated Milk	25.9	0.36	11.5	0.17
Evaporated Milk	25.9	0.36	11.5	0.17
Evaporated Milk	26.1	0.36	11.6	0.17
Average 12 samples			11.9	0.16

The solids content of the original milks (X) was calculated by the following formulas:

Whole milk powders and evaporated milk: $X = \dfrac{0.16S}{C}$.

Skim milk powders: $X = \dfrac{0.16(S-1)}{C} + 3.65$.

In the formulas S and C represent the percentage of solids and citric acid in the milk products; 0.16 is the average percentage of citric acid,

[1] Annual Report No. 37, State of New York, p. 56 (1928).

3.65 is the average percentage of fat in a fluid whole milk, and 1^1 is the average fat content of a commercial skim milk powder.

The calculated solids content of the original milks used in the production of the products listed range from 11.5 to 12.4 per cent, average 11.9 per cent. This average agrees well with that reported for milk by the New York State Department (12.1 per cent). Incidentally it may be mentioned that the chief chemist of one of the large concerns manufacturing milk products informed the writers that in his experience 12 per cent solids is a good average for the milks entering into milk products.

In the last column of Table 2 the percentages of citric acid (X) of the original milks are recorded. The following formulas were used to calculate these values.

Whole milk powders and evaporated milks: $X = \dfrac{12.1C}{S}$

Skim milk powders: $X = \dfrac{C(12.1 - 3.65)}{S - 1}$.

The calculated citric acid content of the original milks used in the production of the products listed ranges from 0.15 to 0.17 per cent, average 0.16 per cent, which average agrees with that obtained on the milks in Table 1.

Based upon the average citric acid value of a milk (0.16%) and the citric acid content of the sample, the milk solids content of an evaporated milk or a sweetened condensed milk may be calculated by the formula, $X = C \times 12.1/0.16$. When this formula was used the three evaporated milks listed in Table 2 were found to contain 27 per cent milk solids. The citric acid content of a sweetened condensed milk was found to be 0.39 per cent. When the above formula is applied the product is found to contain 29 per cent milk solids.

The Federal Standard established the minimum milk solids and fat content of sweetened condensed milk at 28 and 8 per cent, respectively; for evaporated milk the figures are 25.5 and 7.8 per cent. The adoption of the solids and fat requirements of the Federal Standard has led to the established commercial practice of standardization, so that the average fat and solids content (3.65 and 12.1) upon which the calculations proposed are based are not always strictly applicable in all cases. In fact, since the calculations are predicated on a 3.65 to 12.1 fat solids ratio, it is evident that the values obtained by their application are more or less hypothetical. In the case of skimming in the process of standardization an evaporated milk of normal citric acid content would be interpreted as having the full quota of milk solids of average fat content. To be assured that overskimming has not been practiced a fat determination is essential.

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[1] Fourteen samples of commercial skim milk powders examined in the Food Control Laboratory showed an average fat content of 1 per cent.

It is only when the fat content is normal that the procedures for calculating milk solids here described are applicable.

SUMMARY

The data presented show that the average citric acid content of fluid whole milk is fairly uniform (0.16). The calculated solids content of fluid whole milk was found to be 11.9 per cent on the average. Formulas for calculating the milk solids of an evaporated or a sweetened condensed milk are presented. Formulas for calculating the original solids of the milk used in the production of commercial milk products are defined.

ESTIMATION OF MILK SOLIDS IN BREAD

By B. G. HARTMANN and F. HILLIG (Food Control Laboratory,[1]
Food and Drug Administration, U. S. Department of
Agriculture. Washington, D. C.)

The Federal definition and standard for milk bread provides that "the liquid portion of the dough mixture shall consist solely of milk or its equivalent (milk solids and water in the proportions normal to milk)." It is apparent, therefore, that in the preparation of a milk bread any sound milk product or combination of milk products, skimmed or unskimmed, may be employed provided the reconstituted milk used is composed of "milk solids and water in the proportions normal to milk." As a matter of fact fluid milk is seldom used in the preparation of the dough; skim milk preparations and butter fat are usually relied upon as the milk ingredients. At present no reliable procedure for determining the milk solids in bread is available.

The procedure here proposed is based on the solids-citric acid ratio of normal milk and the citric acid content of the bread.

The use of such a ratio assumes that the citric acid content of the dough is derived solely from milk, but this is not strictly true as some of the other ingredients of the dough contain small quantities of citric acid.

Table 1 records the citric acid content in the ingredients which are commonly used in the dough mix and the citric acid of the germ and the bran of the wheat berry. For the determination of the citric acid in these materials the pentabromacetone method was used.

The data given in Table 1 show that of the several non-milk materials in a dough only the malt extract gives a positive test for citric acid by the pentabromacetone method. The largest quantity of malt extract used in the general formula for a dough mixture is about 1.5 per cent, equivalent to 2.5 mg. of citric acid per 100 grams of air-dried bread.

TABLE 1

Citric acid content of bread ingredients and of the germ and bran of the wheat berry.

INGREDIENTS	CITRIC ACID
	per cent
Flour—Soft Winter Wheat	None detected
Flour—Hard Spring Wheat	None detected
Fleischmann Yeast	None detected
Commercial Yeast Food	None detected
Malt Extract Sirup	0.14
Fluid Whole Milk	0.16
Whole Milk Powder	1.29
Skim Milk Powder	1.81
Evaporated Milk	0.36
Wheat Germ	0.34
Wheat Bran	0.08
Whole Wheat Flour	0.05

It will be noted in Table 2 that the "non-milk malt extract" breads contain traces of citric acid as determined by the pentabromacetone method. Under the conditions of the determination approximately 5.0 mg. of pentabromacetone ($130 \times 0.017/0.424 = 5$ mg.) remains in solution in the 130 cc. of reaction mixture. Experience of the writers with the method has shown that at least 1 mg. of pentabromacetone in excess of saturation is necessary to give a positive test (trace) for citric acid. Accordingly, the traces recorded indicate about 2.5 mg. of citric acid ($0.424 \times 6 = 2.5$) in the aliquot from 25 grams of air-dried bread. This quantity is far in excess of that contained in the malt extract used in a dough. Therefore it is concluded that the flour (and possibly the yeast) used in the dough mixture contains citric acid in sufficient quantity to raise the pentabromacetone above the saturation point. Accordingly, in estimating the milk solids in a sample of bread it is necessary to deduct 2.5 mg. from the citric acid determined in the aliquot in order to correct for the citric acid contained in the non-milk portion of the bread.

METHOD FOR DETERMINATION OF CITRIC ACID IN BREAD

To 25 grams of the air-dried bread in a 500 cc. volumetric flask add 100 cc. of water and mix thoroughly. Add 7 cc. of normal sulfuric acid and 200 cc. of 95 per cent alcohol and shake 10 minutes. Then add 5 cc. of 20 per cent phosphotungstic acid solution and shake 5 minutes. Make to mark with 95 per cent alcohol, shake vigorously, and filter through a folded filter paper. Transfer 400 cc. of the clear filtrate to a 16 ounce centrifuge bottle, add 10 cc. of lead acetate solution (75 grams of $Pb(CH_3COO)_2 \cdot 3H_2O$ plus 1 cc. of glacial acetic acid made to 250 cc. with water), shake vigorously for about 2 minutes, and centrifugalize at about 900 r.p.m. for 15 minutes. From this point proceed as directed in the method used for milk.[1] To obtain the grams of citric acid (x) per 100 grams of air-dried bread use one of the following formulas:

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[1] *This Journal*, 15, 644 (1932).

(1) $$X = \frac{1.05(0.424P + 0.000017S) - 0.0025}{16.29} \times 100, \text{ or}$$

(2) $$X = 6.44(0.424P + 0.000017S) - 0.0025,$$

in which P is the grams of pentabromacetone and S is the volume of liquid (cc.) obtained in the filtrate of the pentabromacetone. The numerator in (1) expresses the grams of citric acid corrected for the citric acid contained in the non-milk ingredients (0.0025 gram) and the denominator expresses the grams of air-dried bread in the aliquot:

$$\frac{25}{500 - 9} \times \frac{400}{250} \times 200 = 16.29 \text{ grams, in which 9 represents}$$

the volume of the insoluble solids contained in 25 grams of air-dried bread.

The percentage of milk solids (x) in the moisture-free bread is obtained from the following formula:

$$X = \frac{12.1C \times 100}{0.16(100 - M)} \text{ or } X = \frac{75.6C \times 100}{100 - M},$$

in which 12.1 and 0.16 are the percentages of solids and citric acid, respectively, of fluid whole milk, and M and C are the percentages of moisture and citric acid, respectively, in the air-dried bread.

The solids and citric acid values given in the formula are those used by the writers in the approximation of the milk solids of a milk product by means of its citric acid content (see p. 427).

Table 2 records the percentage of milk solids of authentic breads of known milk solids content.

TABLE 2

Milk solids in authentic milk breads.

MILK PRODUCT IN DOUGH		AIR-DRIED BREAD			MOISTURE-FREE BREAD			
		WEIGHT	MOISTURE	CITRIC ACID	WEIGHT	MILK SOLIDS	MILK SOLIDS	
							ADDED	DETER-MINED
grams		*grams*	*per cent*	*per cent*	*grams*	*grams*	*per cent*	
None		319	6.32	None detected	No malt extract			
None		325	6.28	Trace	Malt extract			
None		342	8.11	Trace	Malt extract			
Fluid Milk,	25.7	342	9.63	0.011	309	3.1	1.0	0.9
Fluid Milk,	51.5	347	7.67	0.026	320	6.2	1.9	2.1
Fluid Milk,	51.5	345	10.02	0.020	310	6.2	2.0	1.7
Fluid Milk,	67.0	337	6.82	0.030	314	8.1	2.6	2.4
Fluid Milk,	105.1	355	6.94	0.047	330	12.7	3.8	3.8
Fluid Milk,	154.5	351	8.02	0.070	323	18.7	5.8	5.8
Fluid Milk,	160.5	359	6.73	0.073	335	19.4	5.8	5.9
Fluid Milk,	206.0	363	9.22	0.086	330	24.9	7.5	7.2
Fluid Milk,	216.2	353	6.39	0.094	330	26.2	7.9	7.6
Fluid Milk,	218.4	372	7.58	0.097	344	26.4	7.7	7.9
Skim Milk Powder,	5.0	341	8.31	0.024	313	6.5	2.1	2.0
Skim Milk Powder,	7.5	344	8.52	0.038	315	9.7	3.1	3.1
Skim Milk Powder,	19.6	367	8.79	0.096	335	25.4	7.6	8.0

The bread samples used were furnished through the courtesy of L. H. Bailey of the Cereal Laboratory of the Bureau of Chemistry and Soils, this Department. The first three samples contained no milk solids, the following 10 samples contained known quantities of fluid whole milk, and the remaining three samples were prepared with skim milk powder. The grams of milk solids (Col. 5) for the breads prepared with fluid whole milk were calculated by multiplying the grams of milk used (Col. 1) by 0.121. The skim milk powder used in the last three breads contained 95.2 per cent solids. One hundred grams of the powder is equivalent to 95.2 − 1 × 100/8.8 = 1070 grams of reconstituted milk of 12.1 per cent solids. Calculated on this basis, the 5.0, 7.5, and 19.6 gram portions of the skim milk powder used in the last three breads are equivalent to 53.5, 80.3, and 209.7 grams of fluid whole milk. The data show good agreement between the milk solids added and the milk solids determined. The method was applied to five samples of the better known breads distributed in the Washington, D. C. market.

TABLE 3

*Milk solids content of market breads.**

BREAD	WEIGHT AIR-DRIED LOAF	AIR-DRIED BREAD		MOISTURE-FREE BREAD MILK SOLIDS	MILK BREAD
		CITRIC ACID	MOISTURE		
	grams	*per cent*	*per cent*	*per cent*	*per cent*
1	323	0.008	6.89	0.6	8
2	455	0.021	8.05	1.7	21
3	320	0.023	9.01	1.9	24
4	338	0.025	13.70	2.2	28
5	300	0.033	6.56	2.7	34
6	342	0.062	14.20	5.5	69

* None of these breads was sold as milk bread.

According to the Federal Standard for milk bread the liquid portion of the dough mixture shall consist solely of fluid milk or of a reconstituted milk of the solids and fat content of normal milk. The general formula for preparing the dough calls for 65 grams of fluid for each 100 grams of flour. Calculated on this basis the percentage of milk solids in a moisture-free milk bread is about 8 per cent.

The data in the last column of Table 3 expresses the percentage of milk bread (X) in the sample, X = 100 M/8, in which M represents the percentage of milk solids in the moisture-free sample and 8, the percentage of milk solids of a bread prepared with fluid whole milk, or its equivalent, as the sole liquid constituent.

It is realized that the results obtained by the method described do not represent the true milk solids because in the calculations it is presumed that the full quota of butter fat normal to milk is contained in the bread.

A bread may show a citric acid content equivalent to 8 per cent whole milk solids and yet lack the butter fat complement. The minimum quantity of butter fat in a milk bread should be 3 per cent.

DETERMINATION OF LACTIC ACID IN MILK AND MILK PRODUCTS

By B. G. HARTMANN and F. HILLIG (Food Control,[1] Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

The method for the determination of lactic acid in milk and milk products here described depends upon the oxidation of the acid to oxalic acid.

In presenting the method the writers are not unmindful of the recognition which the aldehyde procedure for the determination of lactic acid has received from the biochemists. It is, however, quite generally conceded that the procedure is exacting, particularly that part of it which deals with the oxidation of lactic acid to aldehyde.

Friedemann and Graesser,[2] in discussing the technic of the method, state that "it was found that the oxidation to aldehyde appears to depend upon factors such as the concentration of oxidation agent added, the acidity of the solution, the concentration of the $MnSO_4$ and probably also the amount of lactic acid oxidized." These investigators recommend the use of aliquots representing approximately 0.5 cc. of milk and of 0.002 N iodine solution in the titration of the aldehyde.

L. Hart, of this Department, in an investigation (unpublished) of the chemical composition of commercial milk powders, studied the aldehyde method for the purpose of determining its applicability to milk products. His experience was disappointing. Whether the poor results attained were due to adsorption of lactic acid in the casein precipitate or to the difficulties inherent in the oxidation procedure was not determined. However, Hart's work showed that the aldehyde method is not suitable for the examination of milk in food control work.

The oxalic acid procedure is more time-consuming than the aldehyde method, but it has the advantage of simplicity and, furthermore, the quantity of milk which may be used in the determination is not limited, as is that prescribed in the aldehyde method.

Briefly, the method here proposed involves the preparation of the serum, the extraction of the lactic acid from the serum with ether, the oxidization of the extracted acid in alkaline permanganate solution, and the determination of the oxalic acid formed as the calcium salt (titration with permanganate).

[1] W. B. White, Chief.
[2] *J. Biol. Chem.*, 100, 291–308 (1933).

METHOD

REAGENTS

(a) *Casein precipitant.*—Dissolve 4 grams of oxalic acid and 5 grams of potassium acetate in water, add 3 cc. of glacial acetic acid, and dilute to 200 cc. with water.

(b) *Lead acetate solution.*—Dissolve 75 grams of lead acetate—$Pb(CH_3COO)_2$ $\cdot 3H_2O$—in water, add 1 cc. of glacial acetic acid, and dilute to 250 cc. with water.

(c) *Ether.*—Wash ethyl ether three times with water to remove alcohol.

(d) *Sodium hydroxide solution.*—A 30 per cent solution in water.

(e) *Strong potassium permanganate solution.*—A 5 per cent solution of the salt in water.

(f) *Calcium acetate solution.*—A 10 per cent solution of the salt in water.

(g) *Purified asbestos.*—Digest asbestos with the strong $KMnO_4$ (e) in sulfuric acid solution on the steam bath for several hours. Throw the asbestos on a Büchner funnel, and wash with water several times; then transfer to a large beaker and add dilute sulfuric acid and sufficient ferrous sulfate solution to decompose the MnO_2. Wash with hot water until 100 cc. of the filtrate requires not more than one drop of the standard $KMnO_4$ (h) to produce a pink color.

(h) *Standard potassium permanganate solution.*—Dissolve 3.5424 grams of purest $KMnO_4$ in distilled water and dilute to 1 liter. One cc. of the solution is equivalent to 5 mg. of anhydrous oxalic acid.

DETERMINATION

To 50 grams of milk, or 6 grams of a milk powder plus 44 cc. of water, in a 250 cc. volumetric flask, add 100 cc. of hot water (75° C.) and 10 cc. of the casein precipitant (a). Place the mixture on a steam bath for 15 minutes, shaking occasionally; cool, make to mark with water, shake thoroughly, and filter. If the milk does not coagulate, add more potassium acetate and acetic acid. Transfer 225 cc. of the filtrate (disregard slight turbidity) to a 250 cc. volumetric flask, heat to about 50° C. on the steam bath, add 5 cc. of the lead acetate solution (b), shake thoroughly, and return to the steam bath for 30 minutes. Cool, make to mark, and filter. Pass in a rapid stream of hydrogen sulfide, stopper flask, shake vigorously, and again filter. Transfer 200 cc. of the clear filtrate to a 400 cc. beaker and evaporate on a wire gauze to about 5 cc. Pour the contents of the beaker (long-stemmed funnel) into tube "A" of the extractor and rinse with the smallest possible quantity of water. Place the tube in a 1.5 liter beaker of boiling water and evaporate to a volume of about 10 cc. with aeration. (Fit a rubber stopper with a glass tube (3/16 inch bore) long enough to reach nearly to the bottom of the liquid into the neck of the extractor tube and apply suction to the side arm of the extractor.) Rinse the aspirator tube with about 5 cc. of water, add 1 cc. of concentrated hydrochloric acid, and mix. Insert the inner tube "B," connect with the condenser, and extract 3 hours with the washed ether (c). (Use a 20-inch condenser of the Liebig type. Into the receiving flask (an Erlenmeyer of 300 cc. capacity) introduce about 150 cc. of the washed ether. Suspend a piece of strong thread in the ether to assure uniform boiling. The ether should boil as briskly as the conditions will permit (electric hot-plate). Use corks covered with tin foil.)

To the ether extract in the receiving flask, add about 10 cc. of water and expel the ether on the steam bath. Transfer the residue to a 600 cc. beaker with about 100 cc. of water and evaporate on a wire gauze to about 10 cc. Add about 100 cc. of water, 10 cc. of the sodium hydroxide solution (d), and 50 cc. of the strong permanganate solution (e), and heat to about 80° C. Place the beaker in a briskly boiling water bath for 30 minutes. Cool, and add with *constant stirring* ½ cc. less of glacial

acetic acid than is required to neutralize the 10 cc. of sodium hydroxide solution added in the oxidization. Pour about ½ of the mixture into a 250 cc. volumetric flask and pass in hydrogen sulfide until the mixture assumes a yellow color. (The change from yellowish brown to yellow is sudden.) Immediately transfer the remaining portion of the mixture with water to the 250 cc. flask, shake vigorously, and allow to settle. The supernatant liquid, though turbid, should be practically colorless. If yellow, add a few cc. of the strong permanganate solution, mix, and allow to settle. Repeat the treatment until the supernatant liquid is colorless. In case too much permanganate solution was added (pink color), add a small quantity of hydrogen sulfide water, repeating the treatment until the pink color is just discharged. After adjusting for color, cool, mix, make to mark, and filter. Transfer 200 cc. of the *clear* filtrate to a 400 cc. beaker, neutralize with glacial acetic acid (phenolphthalein), and add 5 drops of the acid in excess. Heat to boiling, add 2.5 cc. of the calcium acetate solution (f), boil for a few minutes, and let stand overnight. (The precipitation of calcium oxalate may be expedited in the following manner: Transfer the 200 cc. aliquot to a 500 cc. Erlenmeyer flask. Precipitate calcium oxalate, cool to 15° C., add a small handful of glass beads, shake vigorously for 10 minutes, place in a refrigerator for one-half hour, and again shake for 10 minutes.) Filter the calcium oxalate on a thin pad of the asbestos (g) in a Gooch crucible (removable bottom) and wash with water. Transfer the pad and precipitate to the original beaker, add about 150 cc. of water and 10 cc. of sulfuric acid (1+1), heat to about 80° C., and titrate with the standard permanganate solution (h). One cc. of the standard solution is equivalent to 5 mg. of lactic acid.

Calculate the grams of lactic acid (X) in the portion taken for analysis by the following formula:

$$X = \frac{0.005a}{0.576}, \text{ or } X = 0.00868\text{``a,''}$$

in which "a" is the cc. of permanganate solution used for the titration and 0.576 is the dilution factor $\left(\frac{1}{250} \times \frac{225}{250} \times \frac{200}{250} \times 200\right)$. In arriving at the dilution factor no account is taken of the volume of the casein and manganese precipitates.

DISCUSSION

According to Van Slyke,[1] approximately 20–25 per cent of the entire free lactic acid in a sour milk is adsorbed by casein. In the same bulletin this investigator further concludes that "when milk sours under ordinary conditions, lactic acid is formed, but that this reacts at once, practically as fast as formed, chiefly with calcium phosphate and calcium caseinate, producing monobasic or acid calcium phosphate, uncombined casein and calcium lactate," and that there can not be any considerable amount of free lactic acid until these reactions have been completed.

The chief consideration of any procedure for the determination of lactic acid in milk is the preparation of a serum that is representative of the lactic acid content of the milk. The serum is the casein-free liquid portion of the milk containing lactose, albumin, lactates, citrates, mineral salts, and small quantities of butterfat.

[1] New York State Expt. Sta. Tech. Bull. 140.

PREPARATION OF THE SERUM

As has been observed, the removal of casein tends to adsorb a part of the lactic acid of the milk. After much fruitless experimentation with the customary methods for the precipitation of casein (acids, copper sulfate, etc.) in which adsorption of lactic acid invariably occurred, the procedure described in the proposed method was developed.

The precipitant used in the procedure for the removal of casein contains oxalic acid. Although the use of oxalic acid may appear to be heterodoxy, the fact that the added acid is completely eliminated in a subsequent step fully justifies the procedure. Attempts to show that adsorption of lactic acid does not occur in the procedure for the removal of casein were not entirely successful. However, the results obtained on the recoveries of lactic acid recorded in Tables 3 and 4 show that the adsorption is negligible and that presumably uncombined casein saturated with oxalic acid does not have strong adsorptive powers.

Oxalic acid in weak acetic acid solution precipitates the calcium content of the milk, thereby liberating lactic acid and casein. The addition of the potassium acetate causes the casein to form a coarse grained coagulum, which assures rapid filtration. In addition to precipitating the casein, the treatment with the precipitant removes practically all the butterfat. The removal of butterfat is imperative because both its acid and glycerol components yield oxalic acid in alkaline oxidation.

The second step of the procedure, the treatment of the serum with lead acetate, precipitates oxalic, citric, and phosphoric acids and albumin. The liberation of the lactic acid from its combination with lead is effected with hydrogen sulfide. The solution then contains the lactose and lactic acid of the milk. The lactic acid is isolated from the lactose by extraction with alcohol-free ether, which does not dissolve the sugar.

Extraction of Lactic Acid.—For the extraction of the lactic acid the modified Palkin extractor,[1] illustrated on the following page, was used.

In designing the extractor,[2] the diametrical measurements of tubes A and B were so adjusted that the height of the aqueous head in tube A is about 3 inches when the extractor is in operation. The extractor is a scrubber in effect, approximately 10 liters of ether passing through the highly attenuated volume of solution in 3 hours. As the construction of tube B permits the unobstructed flow of ether, extraction requires very little supervision.

The efficiency of the extractor was tested on aqueous solutions containing 116 mg. of free lactic acid. Recoveries of 95.7, 98.5, 98.3, and 98.5 per cent were obtained in 1, 2, 3 and 4 hours' extraction, respectively. Based upon these results, a 2-hour extraction is sufficient for the recovery of the

quantity of lactic acid contained in a milk that has passed through advanced lactic acid fermentation, and the extra hour assures complete recovery. It was shown by experiment that the ether remaining in the extractor tube was practically free of lactic acid.

As it is quite possible that lactic anhydrides are formed in the course of the procedure, particularly in the evaporations, the following experiment was conducted to determine whether they are soluble in ether. A sirupy lactic acid containing 15 per cent anhydrides, after dilution with water, was extracted 3 hours with ether. A second portion of the solution, after
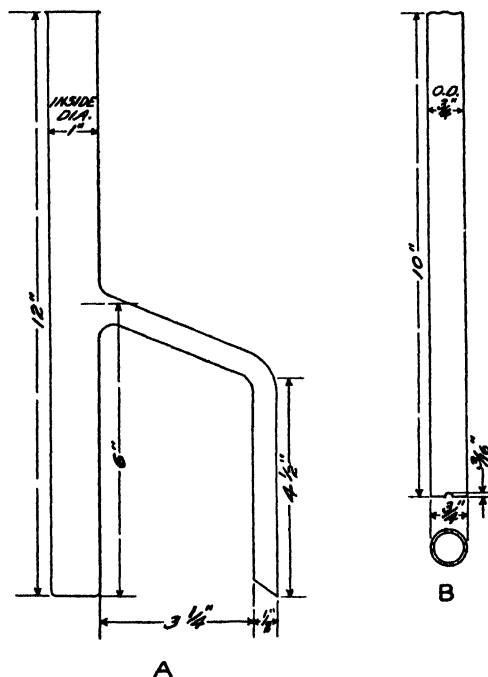
hydrolysis with alkali, was extracted in the same manner. Both extractions recovered about 97 per cent of the added lactic acid, which shows that lactic anhydrides are readily soluble in ether under the conditions maintained in the extraction.

The ether used in the extraction should be free of alcohol, since lactose is soluble in alcohol. The ether extract is practically free of interfering substances; tests for lactose, oxalic acid, and citric acid were practically negative. The results obtained on the synthetic milk (Table 3) confirm this claim.

In some of the ether extractions a small quantity of butterfat was perceptible. One gram of butterfat in direct oxidation yielded only about 10 mg. of oxalic acid. However, it is quite possible that in the treatment of

the serum with lead acetate, glycerine is liberated through saponification. Glycerine is soluble in ether and is oxidizable to oxalic acid. Two grams of a neutral butterfat was allowed to go through the entire procedure. No calcium oxalate was obtained.

Before proceeding with the oxidation, the analyst should remove all traces of ether and alcohol from the solution. This step is important because these compounds produce oxalic acid in alkaline permanganate.

Oxidation of Lactic Acid.—Lactic acid is quantitatively transformed into oxalic acid by oxidation in strongly alkaline permanganate solution—1 mol. of lactic acid produces 1 mol. of oxalic acid.

TABLE 1

Oxidation of lactic acid

SOURCE OF LACTIC ACID	LACTIC ACID IN ALIQUOT		LACTIC ACID RECOVERED
	CONTAINED	DETERMINED	
	gram	*gram*	*per cent*
Lithium salt	0.038	0.037	97
Lithium salt	0.075	0.073	97
Zinc salt	0.090	0.086	96
Lactic acid	0.116	0.114	98

The high returns (Table 1) show that the oxidation procedure is quantitative, and they also demonstrate that the provisions for the removal of manganese and the precipitation of the oxalic acid are satisfactory.

For the oxidation, Bacon and Dunbar[1] recommend heating 30 minutes at 100° C. with 3 grams of sodium hydroxide and 0.75 gram of potassium permanganate. In the proposed procedure the quantity of potassium permanganate was increased to 2.5 grams. This increase does not impair the oxidation, and it assures complete conversion of the lactic acid content of a milk that has gone through advanced lactic acid fermentation.

Removal of Manganese.—Attempts to precipitate the manganese as manganese dioxide by reduction with easily oxidizable compounds, such as alcohols, aldehydes, ketones, etc., showed that their application is invariably accompanied by the production of oxalic acid. In the procedure the manganese is removed as the sulfide. Hydrogen sulfide in alkaline solution reacts with potassium permanganate to form manganous sulfide. An excess of the gas forms alkali sulfides, which on acidification with acetic acid liberate finely divided sulfur, making filtration impossible. This condition is avoided by passing the gas into one-half of the mixture and combining it with the remainder of the untreated solution. The removal of the manganese, if properly done, is not troublesome, filtration is clean and rapid, and there is apparently no loss of oxalic acid through adsorption.

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[1] Bur. Chem. Circ. 78, p. 10.

Precipitation and Determination of Oxalic Acid.—The precipitation of the oxalic acid is made in acetic acid solution, 5 drops of glacial acetic acid in a volume of 200 cc. In the procedure for the precipitation of calcium oxalate the reaction mixture should stand overnight. It was found that by shaking the mixture with glass beads the precipitation can be hastened without detriment to the determination.

TABLE 2

Precipitation of oxalic acid as $Ca(COO)_2$.

SOLUTION CONTAINED	STANDING OVERNIGHT	SHAKEN WITH GLASS BEADS
gram	*gram*	*gram*
0.002	0.003	0.002
	0.002	0.002
0.005	0.005	0.004
	0.005	0.005
0.024	0.024	0.023
	0.024	0.023
0.071	0.071	0.071
	0.071	0.071

Two aqueous solutions (200 cc. each), containing the quantities of anhydrous oxalic acid indicated, were transferred to (1) a 400 cc. beaker and (2) a 500 cc. Erlenmeyer flask, respectively. After approximately 5 grams of sodium acetate and 5 drops of glacial acetic acid had been added, the solutions were heated to boiling, calcium acetate solution was added, and the solution was boiled for several minutes. The portion in the beaker was allowed to stand at room temperature overnight. The contents of the Erlenmeyer flask were cooled to about 15° C.; a small handful of glass beads was added; and the mixture was shaken vigorously for 10 minutes,

TABLE 3

Recovery of added lactic acid in synthetic milk.

	LACTIC ACID		
	ADDED	DETERMINED	RECOVERED
	gram	*gram*	*gram*
The milk		0.004 0.003	
		0.002	
50 grams milk +li-lactate	0.010	0.014	0.011
50 grams milk +li-lactate	0.056	0.056 0.056	0.053
		0.056	

placed in a refrigerator for 30 minutes, again shaken 10 minutes, and immediately filtered on a pad of the purified asbestos. The results show that either method is satisfactory.

EXPERIMENTAL

The proposed procedure was applied to a synthetic milk prepared from casein, lactalbumin, citric acid, butterfat, calcium oxide, and phosphoric acid, in the proportions in which they occur in milk. Table 3 records the analysis of the synthetic milk and of the milk with known quantities of added lactic acid.

The lactic acid indicated in the synthetic milk is due in all probability to small quantities of citric and oxalic acids, butterfat, and lactose, which although insignificant separately, total several milligrams in the aggragate (3 mg. per 50 grams). A correction factor might be applied to take care of the discrepancy, but ordinarily such a provision is not warranted. The returns on the two samples containing added lactic acid are satisfactory although here, as in the experimental data recorded in Table 4, the results indicated are slightly high on low lactic acid and slightly low for the higher quantities.

TABLE 4

Added lactic acid in milk powder.

ACID ADDED	6 GRAMS MILK POWDER*+LACTIC ACID ACID DETERMINED	ACID RECOVERED	LACTIC ACID RECOVERED	
gram	*gram*	*gram*	*per cent*	
0.010	0.026	0.012	120	115
	0.025	0.011	110	
0.026	0.039	0.025	96	100
	0.041	0.027	104	
0.056	0.068	0.054	96	96
	0.067	0.053	95	
0.071	0.083	0.069	97	97
	0.083	0.069	97	
0.119	0.128	0.114	96	96
	0.127	0.113	95	
0.184	0.187	0.173	94	95
	0.188	0.174	95	

* 6 grams of the powder =0.014 gram of lactic acid.

Table 4 presents data obtained on mixtures of a commercial whole milk powder with varying quantities of added lactic acid. Six grams of the milk powder was diluted with water to a volume of 50 cc., and the

lactic acid was added in the form of the sodium salt. Table No. 5 records results of determinations of lactic acid in milk products obtained in the market.

TABLE 5

Lactic acid content of commercial milk products.

KIND OF PRODUCT	LACTIC ACID		
	DETERMINED		IN RECONSTITUTED MILK
	per cent		*per cent*
Fluid Whole Milk (bottle)	0.028 0.029	0.029	0.029
Fluid Whole Milk (bottle)	0.026 0.026	0.026	0.026
Fluid Whole Milk (bottle)	0.024 0.026	0.025	0.025
Fluid Whole Milk (bottle)	0.029 0.028	0.029	0.029
Whole Milk Powder	0.232 0.238	0.235	0.029
Whole Milk Powder	0.217 0.217	0.217	0.027
Whole Milk Powder	0.290 0.260	0.275	0.034
Evaporated Milk	0.078		0.035
Evaporated Milk	0.061 0.052	0.057	0.025
Evaporated Milk	0.084 0.084	0.084	0.037
Sweetened Condensed Milk	0.125		0.050

The data in the last column record the calculated lactic acid content of the original milks used in the production of the milk products listed. The calculations are based on the average milk solids of fluid whole milk (12 per cent); evaporated milk (27 per cent); sweetened condensed milk (30 per cent); and whole milk powder (97 per cent).

The lactic acid content of the four bottled milks ranges from 0.025 to 0.029 per cent, average 0.027 per cent. A sample of milk taken directly from the cow and immediately analyzed showed 0.022 per cent. A number of the milk products listed show a relatively high lactic acid content. It is possible that the lactic acid formed is due to the use of decomposed milk; on the other hand, the high results may be attributable to over neutralization. When milk is overneutralized, lactose is decomposed with

the formation of degradation products which yield oxalic acid in alkaline oxidation. A number of analyses showed that oxalic acid increases with the degree of overneutralization. On the other hand, neutralization does not affect the determination.

TABLE 6

Lactic acid in unneutralized and neutralized milk.

| | LACTIC ACID | | | | TITRATABLE ACID AS LACTIC |
| | UNNEUTRALIZED | | NEUTRALIZED | | |
	per cent		per cent		per cent
Fresh Milk	0.022	0.022			0.19
	0.022				
After 24 hrs. at room temperature	0.057	0.057	0.057	0.057	0.23
	0.057		0.056		
Signs of Curdling	0.133	0.133	0.130	0.131	0.32
	0.133		0.131		
Curdled	0.283	0.287	0.290	0.289	0.53
	0.290		0.288		

The milk was taken directly from the cow. The neutralized samples were prepared by adding sodium bicarbonate in the proportion indicated by the titratable acidity. The data show that neutralization does not affect the determination of lactic acid. The titratable acid was determined with 0.1 N alkali, phenolphthalein being used as indicator. Although the end points in the titrations were not sharp, a fair correlation exists between the increase of acid by titration and determination.

EFFECT OF OVERNEUTRALIZATION OF MILK

When milk is heated with excessive alkali, decomposition products are formed. Decomposition is indicated by the yellow color which the milk assumes. The nature or source of these products was not investigated, but judging from their behavior toward ammoniacal silver, the writers believe that they are aldehydic compounds resulting from the degradation of lactose. The compounds are soluble in ether and, as already mentioned, yield oxalic acid in alkaline oxidation. Obviously, a milk which has been overneutralized when examined by the proposed procedure, will indicate oxalic acid in excess of the true quantity derived from lactic acid itself.

Experiments for the purpose of eliminating the influence of these decomposition compounds, by subjecting the ether extract to a treatment with ammoniacal silver, were not successful. In some instances the treatment had no effect, and in others the effect was pronounced. It is quite possible that the erratic behavior of silver is due to a lack of proper adjust-

ment. However, further work in this direction did not seem to be warranted inasmuch as overneutralization of a milk product is the result of poor factory control; in fact, neutralization in any form is contrary to the accepted practices of the trade.

It is believed that the method described is suitable for determining the lactic acid content of milk in food control work, and that a milk (or milk product) whose lactic acid content on the reconstituted basis is in excess of 0.03 per cent has been prepared from decomposed milk or overneutralized milk.

SUMMARY

A method for the determination of the lactic acid content of milk and milk products in which the acid is oxidized to oxalic acid with alkaline permanganate is described. In this procedure the adsorption of lactic acid in the casein precipitate is reduced to a minimum; manganese is removed as the sulfide; and the precipitation of calcium oxalate is expedited by shaking with glass beads. The liquid extractor developed is highly efficient, permitting the recovery of 98 per cent of lactic acid in less than 3 hours. The results obtained in the investigation show that the method is accurate.

DETERMINATION OF PEROXIDASE IN AGRICULTURAL PRODUCTS*

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In another paper the writers show that hydrogen peroxide may be determined in the presence of pyrogallol and other powerful reducing substances by a modification of the iodometric titration (see p. 395). This permits the estimation of the ferment peroxidase in a new and easier manner, based upon the amount of hydrogen peroxide decomposed by the enzyme.

The method is rapid and comparatively simple, and it has the advantage that the effect of peroxidase may be measured on any substance which is not oxidized by hydrogen peroxide alone, whether this substance gives rise to colored oxidation products or not. There is an added advantage in selecting pyrogallol as the test substance, since, as Willstätter and Pollinger[1] have mentioned, the results are not affected by catalase, which is almost always found in agricultural products. It is thus possible to determine catalase in the presence of peroxidase,[2] and peroxidase in the presence of catalase.

ANALYTICAL PROCEDURE

The method, in detail, is as follows: Enough freshly boiled water is placed in a glass-stoppered cylinder to make a total volume of 250 cc. after the other ingredients have been added. The water is covered several centimeters deep with air-free paraffin oil (vacuum boiled) and placed in a 30° thermostat for 10 to 15 minutes. Then 25 cc. of 0.2 M phosphate buffer (pH = 8.0), 4.0 cc. of 0.1 N hydrogen peroxide (free from preservatives), and 6.25 cc. of 10 per cent pyrogallol solution (= 0.625 g. purest pyrogallol) are added in this order. The contents of the cylinder are stirred or mixed by passing a stream of hydrogen or nitrogen, and the enzyme solution to be tested is then introduced. After the contents have been mixed again, the vessel is returned to the thermostat, and as soon as the oil has collected at the top the first sample of 25 cc. $\left(= \frac{1}{10} \text{ of the total volume} \right)$ is withdrawn in a rapidly flowing pipet. With practice the sample can be taken easily in less than half a minute after the enzyme is added.

The pipet is then allowed to empty into a titrating flask containing 0.5 gram of pyrogallol dissolved in 25 cc. of 2 N sulfuric acid. The moment of half delivery for the first sample is noted on a stop watch and is the starting time of the run. Ten cc. of 10 per cent potassium iodide is added at once, and the flask allowed to stand for not less than 12 nor more than 20 minutes, after which it is titrated with 0.01 N thiosulfate in the usual way. At intervals of 2 and 5 minutes (and perhaps 10 minutes with weak preparations), similar portions are taken for titration.

COURSE OF THE REACTION

Willstätter and his coworkers[1] have repeatedly shown that pyrogallol is oxidized in this reaction to purpurogallin, the amount of the latter being directly proportional to the time of the reaction and to the quantity of peroxidase present. These straight line relationships hold only for a limited range of peroxide concentrations, and for a small amount of purpurogallin formation. The concentrations of peroxide and pyrogallol recommended in this paper correspond closely, therefore, to those chosen by Willstätter and Stoll.[2] The buffer is added because the optimum pH for pyrogallol oxidation is 8.0[3] and the slightly alkaline solution is an advantage when considerable catalase is present. The temperature was placed at 30° instead of 20° because of the expensive equipment needed to maintain a 20° thermostat in most American laboratories throughout the summer.

These changes do not materially alter the course of the reaction, which

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[1] *Loc. cit.*, 400–525.
[2] *Ann.*, 416, 21 (1918). These authors used 5.0 grams of pyrogallol and 50 mg. of hydrogen peroxide in reacting volume of 2 liters without buffer, at 20° C. After 5 minutes the reaction was stopped by adding sulfuric acid, and the purpurogallin was removed by several extractions with ether and determined colorimetrically.
[3] Balls and Hale. Unpublished.

is approximately a straight line for the first 10 minutes, provided the peroxide concentration is low and the amount of enzyme is such that not more than one-fourth to one-third of the peroxide is decomposed. When a is the quantity of thiosulfate used for the titration at 0 minutes, and x that at t minutes, then $\dfrac{a-x}{t}$ represents the decrease in hydrogen peroxide per minute. For a straight line reaction, $\dfrac{a-x}{t}$ is a constant. Table 1 shows that this is approximately true for low peroxide concentrations, but the value varies with the initial amount of peroxide.* Between 0.0016 N and 0.0020 N the variation is slight, consequently small deviations from the recommended amount of hydrogen peroxide are allowable.

TABLE 1

Variation of reaction velocity with hydrogen peroxide.
(250 cc. 0.02 M buffer (pH 8.0) at 30° contained 0.625 gram of pyrogallol, 0.024 mg. enzyme, and peroxide as shown.)

Initial H_2O_2 conc. ×0.001 N	0.44	0.60	0.98	1.40	1.86	2.22	4.46	8.8	13.6	17.1	18.7	44.0
$\dfrac{a-x}{t}$ at 2 min.	0.08	0.09	0.14	0.20	0.25	0.23	0.38	1.0	0.8	0.6	0.3	0.3
5	0.08	0.10	0.15	0.18	0.24	0.25	0.36	0.6	0.6	0.46	0.26	0.3
10	0.06	0.07	0.11	0.17	0.22	0.24	0.33	0.45	0.55	0.32	0.25	0.2

The value of $\dfrac{a-x}{t}$ is also directly proportional to the amount of enzyme present, provided this amount is small enough not to decompose too much peroxide. This is shown in Table 2.

CALCULATION OF PEROXIDASE UNITS

The unit of peroxidase used by Willstätter and Pollinger[1] is based on the "purpurogallin number" (often abbreviated P.Z.). The purpurogallin number is defined by Willstätter and Stoll[2] as the number of milligrams of purpurogallin produced by 1 mg. of enzyme material in 5 minutes

* If the true reaction course of the uninhibited enzyme were linear, then $\dfrac{a-x}{t}$ should remain the same for concentrations of H_2O_2 below 0.002 N. The dependence of $\dfrac{a-x}{t}$ on the initial hydrogen peroxide concentration suggests that the reaction might be monomolecular. For crude and slightly purified enzyme preparations, but not for highly purified ones, the monomolecular formula agrees with the observation better than the linear formula. The monomolecular constant is also proportional to the enzyme concentration, but increases rapidly at peroxide concentrations so small that they are about the lower limit at which accurate measurements can be made.

The fact that $\dfrac{a-x}{t}$ passes through a maximum at about 0.008 N H_2O_2 points to inhibition by excess substrate, as is ordinarily assumed. But the behavior of peroxidase at extremely small peroxide concentrations might, if it could be followed, be found startling. The impression cannot be escaped that the enzyme may always be inhibited in present day tests, so that its true initial velocity may remain unknown but still prove extraordinarily great.

[1] *Ann.*, **430**, 269 (1923).
[2] *Ibid.*, **416**, 21 (1918).

TABLE 2

Variation of reaction velocity with enzyme quantity.

(250 cc. 0.02 M buffer (pH 8.0) at 30° contained 0.625 gram of pyrogallol, 4.0 cc. 0.1 N H$_2$O$_2$, and enzyme as shown. Titrations were made on 25-cc. portions with 0.01 N thiosulfate.

ENZYME QUANTITY	RAW HORSERADISH EXTRACT 0.01 G. PER CC.				MALT SPROUT EXTRACT, DILUTED (0.2 MG. PER CC.)			
	1 cc.		2 cc.		1 cc.		10 cc.	
TIME	cc. THIO.	$\frac{a-x}{t}$	cc. THIO.	$\frac{a-x}{t}$	cc. THIO.	$\frac{a-x}{t}$	cc. THIO.	$\frac{a-x}{t}$
min.								
0	4.00	—	4.25	—	6.70	—	5.90	—
2	3.85	0.08	3.75	0.24	6.40	0.06	3.60	0.46
5	3.60	0.08	3.25	0.20	—	—	—	—
$\frac{a-x}{t}$ per cc. enzyme solution in 5 min.	—	0.08	—	0.10	—	0.060	—	0.046

Crude horseradish peroxidase preparation (0.10 mg. per cc.)

ENZYME QUANTITY	0		0.5 cc.		1.0 cc.		2.0 cc	
TIME	cc. THIO. A	cc. THIO. B	cc. THIO.	$\frac{a-x}{t}$	cc. THIO.	$\frac{a-x}{t}$	cc. THIO.	$\frac{a-x}{t}$
min.								
0	6.05	6.80	5.00	—	5.75	—	5.20	—
5	6.10	6.80	4.40	0.12	4.50	0.25	2.90	0.46
10	6.10	6.90	3.90	0.11	3.45	0.23	1.50	0.37
15	6.10	6.90	3.45	0.10	2.65	0.21	—	—
20	6.10	6.90	—	—	—	—	—	—
$\frac{a-x}{t}$ per cc. enzyme solution in 5 min.	—		—	0.22	—	0.25	—	0.23

Purified horseradish peroxidase preparation (0.012 mg. per cc.)

ENZYME QUANTITY	1.0 cc.		2.0 cc.		3.0 cc.	
TIME	cc. THIO.	$\frac{a-x}{t}$	cc. THIO.	$\frac{a-x}{t}$	cc. THIO.	$\frac{a-x}{t}$
min.						
0	5.30	—	4.65	—	4.40	—
2	—	—	4.15	0.25	3.55	0.42
5	5.10	0.14	3.45	0.24	2.55	0.37
10	4.45	0.12	2.50	0.22	1.35	0.31
$\frac{a-x}{t}$ per cc. enzyme solution in 5 min.	—	0.14	—	0.12	—	0.12

under the conditions of their determination. The peroxidase unit (abbreviated by German writers to P.E.) is the amount of enzyme in 1 gram of material of a purpurogallin number of unity.

$$P.Z. = \frac{\text{mg. purpurogallin formed in 5 minutes}}{\text{mg. enzyme preparation present}}$$

$$P.E. = P.Z. \times \text{grams enzyme material.}$$

It follows that the peroxidase units in a given amount of material equal the grams of purpurogallin produced by this amount of material in 5 minutes reacting time. (Since the peroxidase unit is the quantity of enzyme in 1 gram of material of P.Z. = 1, one unit of peroxidase must be able to produce purpurogallin at the rate of 1 gram in 5 minutes).

The equation for the formation of purpurogallin from pyrogallol by peroxidase action is.[1]

$$2\ C_6H_6O_3 + 3\ H_2O_2 = C_{11}H_8O_5 + CO_2 + 5\ H_2O$$
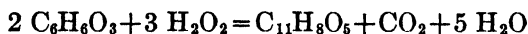
from which $H_2O_2 \times 2.16 =$ purpurogallin. Therefore the quantity (grams) of purpurogallin produced in 5 minutes by the enzyme in the aliquot titrated is $\dfrac{5\dfrac{a-x}{t} \times 0.17 \times 2.16}{1000}$ · So that $P.E. = .00184 \times \dfrac{a-x}{t}$ where a and x are titrations in cc. of $0.01N$ thiosulfate and P.E. = peroxidase units in the *aliquot used for the titration*, namely, 1/10 of the total enzyme used, if the suggested scheme has been followed. It is convenient to express peroxidase quantities in peroxidase units per gram or per cc., remembering that the quantity units per gram is also the purpurogallin number.

The value of $\dfrac{a-x}{t}$ may be calculated separately from the titration made at 2 minutes and that at 5 minutes. They should check. A 10-minute titration may be also used when the previous decrease in peroxide has been small, and there is no objection to continuing the titrations as long as $\dfrac{a-x}{t}$ remains constant within the error of the titration. When this value decreases, it indicates that the enzyme has been decomposed by the peroxide.

The peroxidase content of an enzyme material obtained in this way will not be the same as that obtained by the method of Willstätter and Stoll, because of the differences in pH and temperature between the two determinations. The method described here gives results about twice as large. If it is desired, the results may easily be obtained in Willstätter units by omitting the buffer from the set-up, and running the determination at 20°. The iodometric titration is quantitative in either case.

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[1] *Ann.*, **433**, 17 (1923).

EFFECT OF CATALASE

The measurement of peroxidase action in an excess of alkaline (*pH* = 8) pyrogallol avoids any interference due to the presence of catalase. Since catalase also decomposes hydrogen peroxide, its action could be confused with that of peroxidase under some conditions. The catalase liberates molecular oxygen, but this is at once reabsorbed by the pyrogallol and again forms a decomposable peroxide. Since the amount of pyrogallol is large in comparison with that of the peroxide, and the catalase is rapidly inactivated at 30° in any case, this reaction is quantitative, so that the hydrogen peroxide available for peroxidase action and for the iodine titration remains constant and unaffected by relatively large amounts of catalase. The following experiment on a peroxidase from horseradish and a catalase preparation after K. G. Stern[1] illustrates this.

TABLE 3

Effect of catalase on peroxidase determination—(pH =8.0 temp. 30°, pyrogallol, 0.625 gram per 250 cc.).

TIME	CATALASE ONLY 0.5 CC. NO PYRO.		CATALASE ONLY 0.5 CC. +PYRO.		CATALASE 0.5 CC.+ +PEROXIDASE 0.10 MG. +PYRO.		PEROXIDASE ONLY 0.10 +PYRO.	
	CC. THIO.	$\frac{a-x}{t}$	CC. THIO.	$\frac{a-x}{t}$	CC. THIO.	$\frac{a-x}{t}$	CC. THIO.	$\frac{a-x}{t}$
min.								
0	4.70	—	5.80	—	5.60	—	6.05	—
5	2.87	0.37	5.80	0	4.25	0.27	4.60	0.29
10	2.00	0.27	5.75	0	3.15	0.25	3.55	0.24

In thus replenishing the peroxide decomposed by catalase, some of the pyrogallol is oxidized, so that after peroxidase-free catalase is added to the pyrogallol-peroxide mixture it turns brown, although the peroxide titration remains unchanged.

Alkaline pyrogallol also reduces atmospheric oxygen to hydrogen peroxide. It is, therefore, necessary to exclude air from the reaction, otherwise the peroxide content of the solution will increase. The layer of oil over the pyrogallol solution protects the latter from the air, but it seems almost impossible to avoid introducing traces of oxygen during the mixing of the solutions as well as dissolved in the buffer and hydrogen peroxide added. For this reason the iodometric titrations are always slightly higher than the equivalent of the added hydrogen peroxide. When care is taken to minimize this effect, it has no serious results, since at the concentration of hydrogen peroxide selected small variations in this do not greatly influence the rate of oxidation.

Method for Agricultural Products.—The method for handling the enzyme solution has already been described. First, however, it is necessary to extract the enzyme from the raw material.

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[1] *Z. physiol. Chem.*, 204, 259 (1932).

Five grams of the water-containing material to be analyzed is ground fine in a mortar with clean sharp sand, and 45 cc. of 0.1 M phosphate buffer (pH = 8.0) is added gradually. This gives a suspension containing 1 gram of original material in 10 cc. The sand and larger particles are then settled or centrifuged out, and a suitable portion of the supernatant liquid (usually 1 or 2 cc. with peroxidase-rich plants) is used in the enzyme determination. For dry materials, a better proportion is often 1 gram to 49 cc. of buffer.

Such an extraction is practically complete, even when the suspension is centrifuged until free from all solid matter. This is seen by comparing the total peroxidase obtained by exhaustive extraction of the material with the total obtained by calculating from the aliquot of the first extract, as above.

TABLE 4

Showing that the extraction of peroxidase is nearly quantitative

PEROXIDASE UNITS	HORSERADISH	TURNIPS	RADISHES
Removed by first extraction	1.56	0.47	0.124
Removed by second extraction	0.24	0.04	0.008
Removed by third extraction	0.06	0.01	—
Removed by fourth extraction	0.03	—	—
Remaining in residue	0.24	0.05	0.012
Total	2.13	0.57	0.144
Determined in first extract and calculated to total volume of suspension (= first extract + sediment).	2.03	0.55	0.138

OCCURRENCE OF PEROXIDASE

Peroxidase occurs chiefly in plant roots, notably in horseradish and turnips. Other plant tissues contain the enzyme in smaller amounts, but the sprouts, particularly the root sprouts of grain, are exceptionally rich.[1] The peroxidase develops just prior to the visible sprouting of the grain and thereafter remains comparatively constant.

Peroxidase, therefore, is to all appearances less widespread than catalase. This ferment is, however, a good example of the general principle that the presence of an enzyme can be proved only by its activity, whereas its absence cannot be proved at all, for it may be present but inactive. It is evident that naturally formed inhibitors frequently accompany peroxidase. In horseradish and turnips such inhibitors are probably at a minimum, but in malt sprouts, for example, they are present in quantity. This is indicated by the fact that peroxidase activity usually increases on purification, although it is certain that some enzyme is lost. It is also shown by the fact that sprout extracts, prepared as directed,

[1] Untersuchungen über Enzyme, I, 521 (1928).

do not give a constant reaction velocity. Instead the enzymic activity decreases until after 10 or 15 minutes it has completely disappeared. A similar effect may be artificially produced with pure preparations by adding traces of chemicals, such as para amino acetanilide. The inactivation is produced only during the course of the ferment action and not by pretreatment, and it is therefore seemingly due to an acceleration of the inactivation by peroxide. The peroxidase content of a natural product, such as malt sprouts, which contains an inhibitor is therefore difficult to estimate. Efforts to destroy the inhibitor while saving the enzyme have been unsuccessful, nor is it possible to extrapolate observed results to zero time, and so arrive at a value for the initial velocity. The curve of velocity change approaches the axis asymptotically. For practical purposes the best method seems to be to use unusually small amounts of enzyme for the determination, thereby diluting the inhibitor as much as possible.

In Table 5 are listed peroxidase determinations made by the method here described on a number of common agricultural products. They only indicate the order of the peroxidase content, since no attempt was made to secure representative samples. The materials marked with an asterisk (*) also contained appreciable amounts of inhibitors, and the determinations are, therefore, less accurate.

TABLE 5
Approximate peroxidase content of some plant products

	PEROXIDASE UNITS PER KILO		PEROXIDASE UNITS PER KILO
Malt sprouts*	550	Beets	11
Horseradish	403[1]	Wheat*	10
Turnips	110	Barley	9
White potatoes	36	Barley with hulls	3
Sweet potatoes*	30	Oats	3
Radishes	30	Onions	3
Rye	14	Soybeans	2

The grains were dry, the vegetables not dried. Except radishes and onions, the plants had been stored through the winter.

[1] As an example of the calculations, 5.0 grams of fresh horseradish was treated as described with 45 cc of buffer solution. One cc. of the resulting milky fluid was diluted to 10 cc. with water, and 1 cc. of this dilution was added to a 250-cc. determination. From this, 25-cc. portions were removed for titrations. The amount of horseradish present per titration was thus 1 mg. After 5 minutes a second titration showed a decrease of 1.10 cc. 0.01 N thiosulfate. Thus $\frac{a-x}{t}$ =0.22; 0.22 ×.00184 =.000405 peroxidase units in 1 mg., or 0.403 units per gram.

PURIFICATION OF PEROXIDASE

Willstätter and Pollinger[1] have concentrated peroxidase as much as 12,000 times. Such preparations are naturally difficult to make. Following the suggestions of Willstätter regarding concentration by means of adsorbents, however, it is easy to make a peroxidase which, while not at all

[1] Untersuchungen über Ensyme, I, 521 (1928).

comparable to the classical preparations, is nevertheless suitable when a moderately pure peroxidase is required.

The method depends upon the extraction of the ground horseradish with dilute sodium bicarbonate, the adsorption of the enzyme from this solution on alumina A, and its subsequent elution with carbon dioxide. The following experiment is cited as an example: 1.0 kg. horseradish roots was cut into thin lateral slices and dialyzed in running tap water for 8 days. The slices then contained 575 units of peroxidase. They were ground in a Wiley mill, with the continuous addition of enough 2 per cent sodium bicarbonate solution to produce a rather thin paste. This was filtered and the pulp mixed twice more with sodium bicarbonate solution and filtered off. The combined extracts were mixed with an equal volume of 95 per cent alcohol, and the resulting precipitate was removed with kieselguhr.

By this time the liquid was definitely alkaline to litmus, and was reneutralized by the addtion of a little dilute acetic acid. The peroxidase was then precipitated by adding three volumes of 95 per cent alcohol to the neutral solution. The precipitate was filtered out, again with the aid of kieselguhr, as it is sticky. The kieselguhr was extracted with 1 liter of water and then filtered out. The resulting dark-brown liquid measured 1000 cc. and contained a total of 183 units, a yield of 32 per cent. The enzyme was again precipitated by the addition of 6 volumes of cold 95 per cent alcohol to the cold solution. The precipitate was allowed to settle, filtered off, washed twice with acetone and once with anhydrous ether, and dried. It weighed 8.06 grams and contained 126 units or 15.6 units per gram.

Five-tenths gram of this preparation (7.8 units) was dissolved in 4.5 cc. of water, and poured into 5.5 cc. of alcohol. The precipitate which formed was centrifuged out and discarded. The supernatant liquid was then adsorbed five times with 10 cc., 5 cc., 5 cc., 5 cc., and 2.5 cc. of Alumina A suspension[1] containing 55 per cent alcohol by volume. The supernatant liquid after the last centrifuging was almost colorless. The combined sediments were washed with 25 cc. of 55 per cent alcohol, re-centrifuged and suspended in 53 cc. of water. After the solution had been cooled in an ice bath, CO_2 was passed through for 30 minutes, the alumina was removed in the centrifuge and the supernatant solution of enzyme freed from CO_2 by the vacuum pump. The volume was 50 cc. The solution contained a total of 6.3 units. On evaporating 2.00 cc. of the first solution to dryness, a residue of 3.4 mg. was obtained, of which 1.4 mg. was ash. The organic matter per cc. of enzyme solution was, therefore, 1.0 mg., so that the preparation on a dry basis contained 119 units per gram, a concentration of about 200 times, with a yield of 22 per cent. Similar preparations were purer than this, but the analytical data on the intermediate steps were not secured.

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[1] *Ber.*, **64**, 1697 (1931).

BOOK REVIEW

Qualitative Chemical Analysis. *Certain Principles and Methods Used in Identifying Inorganic Substances together with a Systematic Survey of the Chemistry of these Materials.* By Roy K. McAlpine, Ph.D., and Byron A. Soule, Sc.D., University of Michigan. Based upon the text by A. B. Prescott and O. C. Johnson. D. Van Nostrand Company, Inc., New York, 1933. Cloth $4.50.

For more than 60 years "Prescott & Johnson" has been a familiar reference work to most American students of qualitative analysis, and many analysts also have had an intimate acquaintance with it. The continued popularity of the book is attested by the necessity for its revision and rewriting a quarter of a century after the death of its original authors and 17 years after its revision by Prof. Olsen. Discoveries in general chemistry that have a bearing on qualitative analysis have been numerous since 1916, and the literature on the subject has increased enormously. This new matter has been carefully examined and the worth-while discoveries embodied in the revision. This work was begun in 1864 by Professor S. H. Douglas. It was in the form of Tables for Qualitative Analysis, which were prepared for use in connection with Fresenius' Manual of Qualitative Analysis. These Tables passed through three editions, after which they were incorporated in the first edition of Douglas and Prescott's Qualitative Chemical Analysis in 1873. This work reached three editions, and in the fourth (1883) it appeared under the name of Prescott and Johnson's Qualitative Chemical Analysis.

The most important changes in the new book as compared with the 7th edition are in Part I. The discussion of the classification of the metals as bases, operation of analysis and solution, and ionization occupy but 26 pages in the 7th edition whereas in the new revision Part I occupies 148 pages. In this part there are now discussed such topics as aims of qualitative analysis, requirements of group reagents, the theory of reversible reactions, the reactions of inorganic substances, the application of chemical theory in qualitative analysis, the solubility problem in analytical chemistry and the delicacy of color reactions of the metals. Although much of this is elementary physical chemistry, its careful study is essential to an understanding of the laws underlying the science of qualitative analysis. This should prove especially helpful to the student of the subject who has not taken physical chemistry. The remainder of the book follows in general the scheme and style of the older editions but new tests, and elaborations of the older ones, have been added as discoveries warranted. Great advances have been made in the application of organic substance to the detection of the metals. Many of these are mentioned even though the reagent is not sufficiently common for routine laboratory use. For example, four qualitative tests with organic substances are given for magnesium, five for nickel, and four for zinc. It is unfortunate for science that such terms as "soluble," "slightly soluble," "insoluble," etc., still remain purely arbitrary. The values assigned to these terms by McAlpine and Soule are much different from those given in the U. S. Pharmacopoeia. In addition to being a guide in qualitative analysis, information concerning the properties of metals and their salts is given in great amount and variety throughout the work. This places the book in the semi-encyclopedic class. Analysts and students of qualitative analysis should have ready access to the book. Many will wish to own it. Naturally, teachers and analysts who have cherished their copies of "Prescott & Johnson" since their student days will want it because of the wealth of new material which it contains.—L. E. WARREN.

TUESDAY—MORNING SESSION

THE NEVER-ENDING PROBLEMS OF THE REGULATORY CHEMIST[1]

By P. B. DUNBAR (Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

On reflection it has seemed to me that the title of this paper, "The Never Ending Problems of the Regulatory Chemist" is a misleading one. I do not mean to infer that the regulatory chemist never solves his problems. What I want to discuss is rather, "The Ever-Changing Problems of the Regulatory Chemist."

You have heard of the Irishman who obtained employment as a farm hand. He was set to plowing a field. To aid him in drawing a straight furrow his employer directed him to keep his eye fixed on a distant object. The Irishman carried out his instructions to the letter but when the farmer returned he found that the furrow had wandered over the entire field. When asked why he had not kept his eye fixed on a distant object the plowman replied, "Bejabers and I did, but the blamed cow wouldn't stand still."

From the day when the first regulatory alchemist fixed his eye on a regulatory cow until this very hour the objective has failed to stay put. What is worse, the cows have a habit of multiplying, as cows will, until the modern chemist engaged in law enforcement work is hard put to it to select the particular objectives most deserving his attention.

In the early summer of the present year, the Food and Drug Administration found itself confronted with one of its many new regulatory problems. Certain spice importations received at our principal ports had been found to contain mouse excreta. The Administration's port laboratories promptly began detentions of the adulterated material. No better method of detecting the adulteration appeared available than the laborious one of picking out the objectionable material by hand. The regulatory problem is happily solved. Importations are now very generally clean. One incidental by-product of this campaign, however, is particularly pertinent to this discussion. It stimulated the inventive genius of a tired chemist who swung into action with his pencil and produced a drawing, a la Rube Goldberg, entitled "The Preliminary Examination for Mouse Excreta." The apparatus consists of a cage containing an alert looking feline, with neck and tail wired to a galvanometer. An equally alert analyst observes the instrument, at the same time agitating a bag of the suspected seeds before the feline nose. A legend states: "If cat shows ex-

citement as indicated by the movement of the galvanometer, mouse excreta are present." This promising method, submitted wholly unofficially I hasten to add, was returned to the author with a compliment on its ingenuity and the comment that he had opened a new and promising field of biochemistry. It was suggested that the method be submitted to the Association of Official Agricultural Chemists. The author was criticized, however, for rushing into print without first completely exploring the possibilities of his method. It was pointed out that his description was lacking in detail, and that he had totally neglected the opportunity of making his method quantitative. Such is the usual attitude of critics when dealing with new scientific methods. As was often pointed out by my old teacher of physical chemistry, Professor Harry C. Jones, the ordinary comment in the case of a new chemical discovery is, first, it is not true; second, it is not new; third, it is no good anyhow.

My story illustrates two points entirely pertinent to my subject—first, the eternal variety of the technical problems confronting the regulatory chemist; second, the unfailing urge for the exercise of ingenuity in devising new methods of attack. It has been my observation that research chemists divide themselves very roughly into two classes, although the dividing line is by no means sharp. In one group the urge for research has its origin in an overwhelming curiosity and a commendable desire to accumulate scientific facts without any particular interest in their practical value and immediate utilization. Research workers of this kind find their most congenial atmosphere in university laboratories, although this type is much less prevalent there than was the case a generation ago. The second group of research workers is distinctly utilitarian in its outlook. It is interested in collecting scientific facts but has little use for them unless they can be turned to a fairly immediate practical end by the development of processes and methods. The second group comprises the factory chemists, scientific directors of industrial enterprises, and regulatory chemists. The work of the regulatory chemist is intensely practical. The diversity of the problems daily encountered, the urge of new discovery, the excitement of following the trail of the violator, the appeal of the work from a public service standpoint, combine to make a composite of absorbing interest.

My introduction to regulatory work occurred twenty-five years ago when the Bureau of Chemistry was just entering upon the enforcement of the food and drugs act. My acquaintance with this Association began at the fall meeting of 1908. Food chemistry in those days was a neglected subject in the average university course. The young student was fortunate indeed if his school experience in food analysis extended beyond a few determinations such as butterfat and protein in milk. Such an individual suddenly confronted with the regulatory problems of that day involving foods and drugs could not help but be overwhelmed. To the

young chemist just entering the regulatory field, Bureau of Chemistry Bulletin 107, containing the compiled methods of the Association of Official Agricultural Chemists, seemed to offer everything that could be desired. That bulletin, just off the press, superseding Bulletins 46 and 65, appeared to the unpracticed eye to contain all the methods that could be needed to solve the regulatory puzzles then surrounding us. Disillusionment came quickly. It soon became evident that many of the impressive procedures listed in Bulletin 107 were distinctly inefficient. A most discouraging feature was the frequency with which the thoughtful chemist was obliged to admit that his results obtained by these official methods were unsatisfactory and inconclusive and would not stand the fire of attack in court. This was true even in the case of methods designed to detect some of the more classic forms of food adulteration. Many of these classic types of adulteration, so effectively and justly used to arouse the public and through them the Congress and various State legislatures into passing remedial legislation, rapidly went out of fashion because they could not long withstand the assaults of regulatory operations, but food and drug adulteration did not go out of style. With the advent of more effective enforcement twenty-five years ago, it became immediately obvious that better methods for the detection of the surviving old forms of adulteration and new methods to cope with modern and more insidious forms must be developed without delay. I recall distinctly that although food preservatives were at the moment an issue of first importance, the chemist was handicapped by an almost entire lack of exact quantitative methods for the determination of substances like benzoic acid.

It is to the credit of the regulatory chemist and particularly of this association that the past twenty-five years have been a period of continual progress. Bulletin 107 was itself a marvelous advance over its predecessors. A comparison between Bulletin 107 and the present official book of methods is illuminating. Bulletin 107 contained just 230 pages of text and 30 chapters, several of which were briefer than the legendary chapter on snakes in Ireland, consisting of the terse phrase, "In preparation." Our current book of methods contains 564 pages, comprising 42 chapters, a number of which it must be admitted are also in the "In preparation" class. Bulletin 107 contained two pages devoted to drug analysis as compared with 52 in our current official book. The chapters of our present book of methods contain analytical procedure not even dreamed of when Bulletin 107 was compiled.

Bulletin 107 was a creditable piece of work, just as our present book is a credit to the association. There is, however, no more reason for complacency today than there was in 1907. The task of the regulatory chemist is ever changing and never ending. It is a trite statement that the adulterator is one jump ahead of the regulatory chemist. With increasing knowledge of the composition and properties of foods and drugs we recog-

nize today more clearly than ever before, the existence of certain prac-
tices in the industries constituting distinct violations of law which per-
haps were not appreciated as such, either by manufacturers or by regu-
latory officers a decade ago.

I hardly need cite to this audience the problems which the regulatory
chemist has met and overcome during the past quarter of a century. The
estimation of fruit content and added pectins in jellies and jams, the
identification and estimation of the organic acids, the quantitative de-
termination of egg solids in foods, and innumerable other chemical
obstacles have been overcome and no longer present serious difficulties.
We are more concerned, however, with problems that are now before us
pressing for solution.

In our regulatory operations in the Food and Drug Administration we
are accustomed to class violations of the food and drugs act involving
foods in three groups; first, those violations distinctly involving public
health such as the food product containing an added poisonous ingredi-
ent; second, products in which the violations constitute offenses against
decency, such as decomposed or filthy articles; third, economic cheats not
affecting public health but distinctly fraudulent and therefore affecting
the public welfare. Today as never before, we are recognizing the crying
need for accurate methods to aid in establishing without fear of con-
tradiction in the courts, the presence of dangerous amounts of poisonous
materials. As a collateral problem we appreciate the need for pharma-
cological experimentation to establish the degree of toxicity of these ma-
terials, which in minute amounts may lead to such serious chronic effects.
At this very moment we are confronted with a most serious question re-
garding the use of fluorine sprays as insecticides on fruits and vegetables.
Unmistakable evidence is at hand pointing to the danger of minute
amounts of fluorides in food products. Extensive work must still be done
to determine whether or not there are any safe limits for this substance.
Before progress can be made in this direction the chemist must be pre-
pared to estimate fluorine with the utmost precision. Our current methods
for this determination are wholly inadequate. I am glad to say that the
Food Control Laboratory of the Food and Drug Administration is making
excellent progress in developing an analytical method. As you are aware,
fluorine will be the subject of discussion at one of the sessions of this con-
vention. Certainly before these problems are satisfactorily solved, others
equally pressing and important will confront us.

In the second group of violations involving offenses against decency,
we are in serious need of prompt development of exact methods for the
estimation of filth and decomposition. Chemical methods in this direction
today are far from adequate. New and original chemical procedure must
be devised. The bacteriologist and the microanalyst have made important
contributions to this field but both are handicapped in numerous par-

ticulars. Perhaps an even broader field for the ingenuity of the regulatory chemist is to be found in the development of new methods for detecting economic cheats. Let me quote from the Annual Report of the New York State Department of Farms and Markets, for 1926. In that portion of the report devoted to the chemical laboratory appear these words:

While it is true that the grosser forms of adulteration and misbranding are disappearing, it is equally true that modern commercial enterprise is constantly seeking to introduce new foods and food preparations and new combinations of foods, and in some cases to incorporate into foods new synthetics and other non-foods of dubious physiological effect. In these days of high-pressure salesmanship and keen competition, the food manufacturer, however high his ethical standards, cannot be humanly expected always to protect the consumer from deception on the one hand and the rival manufacturer from unfair competition on the other. This is the legitimate province of the food control chemist and he was never more needed than to-day. It is one thing to detect such gross and obsolete forms of adulteration as lamp black in black pepper and talcum powder in confectionery, and quite another to cope with the subleties of present-day food sophistication.

Discussing then the food analytical problems of the day, the report continues:

Next to oils and fats, the "unscrambling" of sugar mixtures is one of the hardest tasks of the food chemist.

The writer then goes on to cite other current problems such as the determination of vitamins, the identification and estimation of synthetic flavors, of alcohol substitutes, and of "emulsifiers." A hasty review of the program of this meeting will show that these analytical puzzles and others like them are still with us and no doubt many of them will continue to confront us indefinitely in the food field.

In the drug field there is a crying need for methods useful in the identification and estimation of glandular ingredients. Gland therapy is today a popular plaything of the medical profession and glandular products are a rich field for the quack manufacturer. We must have methods which will aid us in attacking this regulatory problem. The perfection of bioassay methods for many vital drug products lies far in the future. Material progress must be anticipated if we are to meet our regulatory obligations. The list might be expanded indefinitely. That section of *Industrial and Engineering Chemistry* for October, 1932, known as The Listening Post, makes this significant statement:

When advertising agencies discovered that science and scientists had a public appeal in their promotional campaigns, trouble started for all concerned. Not only has the general public been given a most unfortunately warped impression of the keen interest of chemists, physicians, dentists, physicists, and others, in cigarettes, tooth pastes, toilet papers, and a host of other common commodities, but also the license taken in stating research findings frequently presents a false picture of their real nature. When discussion of this subject is as general as at present, it is refreshing to get the real facts in at least one moot case.

The comment just quoted refers to an article in the same issue by Gordon and Shand on the "Surface Tension of Tooth Paste Solutions." The authors of this excellent paper confess that the studies reported were "undertaken to knock down the 'scientific straw men' created by dentifrice advertising." Unmistakably the future regulatory chemist will find himself confronted with these pseudo-scientific straw men in both food and drug fields to a degree calling for his best efforts if he is to deliver an effective knockout punch.

It is most unfortunate that this Association, because of its somewhat loose organization and the fact that its workers are serving voluntarily, has been unable in the past to operate in a more systematic fashion. It has long been my feeling that our progress has been distinctly handicapped by lack of organization. In his presidential address delivered at the Fortieth Annual Convention, on October 21, 1924, my beloved friend, Mr. R. E. Doolittle, who contributed so much to the Association, presented a thoughtful study of this subject under the title "The Needs of our Association." Mr. Doolittle stated among other things:

The most important problem that confronts the Association as I study the situation is a proper coordination of its activities. Little difficulty is experienced in securing competent chemists to take charge of its investigative and collaborative studies, but a referee or associate referee, once selected, is left almost entirely to his own resources. Those of you who have served on Sub-committees A, B, and C well know how, in many instances, referees have taken up their work without consideration of the recommendations made or of the work done by former referees. Our proceedings are replete with reports on lines of work well begun but never finished. Again, retiring referees have made no recommendations for further work, and when new referees are in doubt as to what are the most important problems to take up, no one is designated to assist them. On the other hand, some referees have felt that they were restricted in their activities to the recommendations of the former referee, although there were, in their own opinion, more important lines of work which should be considered. Then, too, referees have taken up lines of work without consideration of methods already adopted by the Association or of work already done and reported upon. Perhaps some of those unsatisfactory conditions have been due to a lack of proper cooperation by the officers of the Association or to failure to publish promptly the reports and recommendations of referees, but they are for the most part, in my opinion, due to a faulty plan of operation. No method is provided whereby the referee or associate referee shall notify or even consult with any committee or officer of the association concerning his plans for work at the beginning of his term of appointment or of the progress he is making during the year. Often the first and only information that the Committee on Recommendations of Referees receives concerning the line of work a referee is engaged in, is the referee's report, which comes to hand a few days before the annual meeting, and failure to receive a report is often the first and only information the committee receives that a referee has not been active during the year.

Mr. Doolittle was discussing a condition which had given him concern for several years. I had the pleasure of cooperating with him the previous year in formulating a plan, which, in a subsequent portion of his address, he described in the following terms:

During the past year your officers endeavored to devise and to put into operation such a method, which for want of a better name, we designated as the project plan of operation. The principal features of the plan are as follows: (1) All work on methods for a product or for a group of similar products, as usually represented by a chapter in the Book of Methods, shall be placed under the direction of a referee, designated as general referee, who shall be responsible for all the methods under his subject and each line of work under a general subject shall be placed in the hands of an associate referee, who advises with, and reports to, the general referee. (2) Each associate referee shall immediately after acceptance of appointment submit to the general referee an outline of the work he proposes to do during the year, this outline to be based upon the recommendations of the previous referee, as considered and reported upon by the Committee on Recommendations of Referees and approved by the association. (3) The general referee shall consider the plan submitted by the associate referee, particularly for proper coordination with the work of other associate referees on his subject, existing association methods, work previously done, etc., and shall notify the associate referee of his approval of the plan submitted or of any modifications deemed necessary. The general referee shall also submit to the Chairman of the Committee on Recommendations of Referees an outline of the final plans for work agreed upon for the associate referees on his subject. Similarly, referees operating independently shall notify the Chairman of the Committee on Recommendation of Referees of their plans of work for the year. (4) Each associate referee shall submit a brief report of the progress of his work at quarterly intervals to the general referee under whom he operates, and the general referees and independent referees shall in a similar manner make brief reports of the progress of the work on their respective subjects at quarterly intervals to the Chairman of the Committee on Recommendations of Referees.

At the time this project plan was devised it was written up in detail, in a two page memorandum, and distributed to the various referees. I am led to believe that the plan has been almost entirely neglected. Unmistakably if it were followed the work of this Association would be materially enhanced. I appreciate, however, the impossibility of attaining such an ideal form of organization in an association in which the workers are making entirely voluntary contributions. I venture to express the hope that the time may come when the Association can afford to employ several technically trained individuals who can maintain actual supervision of an active kind over the work of the referees. Until that ideal situation is attained, however, I make bold to suggest that according to my observation many referees and associate referees are wholly unaware of their obligations when they accept a refereeship. They are further unaware of their rights as referees. It is my recommendation that steps be taken by the Association at the time an individual is offered and accepts appointment as a referee or associate referee, to make clear to him in unmistakable terms that he is, during the period of his tenure of office, responsible for all the methods connected with his particular subject, that he must consider the recommendations of his predecessor, and if these have been acted upon affirmatively by the Association, must conform with these recommendations unless specifically excused, upon appeal to the appropriate committee chairman or group referee; that in

addition to following out the approved recommendations of his predecessor he is at liberty to engage in other investigations of a pertinent character within the field of his refereeship, that it is his obligation to develop effective new methods for subjects within the scope of his assignment and to modify existing methods and that he is also expected to recommend the deletion of obsolete or inaccurate methods. If, as I am firmly convinced, the task of the regulatory chemist is to become an increasingly difficult and exacting one, this Association can make its full contribution only by adopting a systematic policy in the control of its referees.

REPORT ON PLANTS

By O. B. WINTER (Agricultural Experiment Station,
East Lansing, Mich.), *Referee*

In the reports on plants last year it was recommended that further study be made on the preparation of plant material for analysis and on methods for the determination of chlorine, fluorine, sodium, less common elements, carbohydrates, and different forms of nitrogen in plants. Associate referees were appointed for all these problems with the exception of methods for the determination of fluorine and sodium. The progress made by these associates will be presented in their respective reports and the referee concurs in their recommendations.

All the available time that the referee could spend on the work of the Association during the past year was given to a study of fluorine. Several methods were studied, and a paper describing one of these has been accepted for publication.[1] Undoubtedly the striking feature of the method is the fact that fluorine may be volatilized from most of its compounds by simply placing the sample, together with perchloric acid, water, and a few glass beads or porous plate, in an ordinary distillation flask and boiling at a comparatively low temperature. The fluorine in the distillate may then be titrated with standard thorium nitrate. A resumé of the method will be given on Wednesday morning at the symposium.[2]

No work was done during the year on methods for the determination of sodium in plants. An associate referee should be appointed for this problem.

The Associate Referee on Preparation of Plant Materials for Analysis has devised satisfactory methods for the determination of mineral constituents and carbohydrates. His opinion is that it is not feasible to do work on the preparation of these materials for the determination of different forms of nitrogen until those methods are more fully available. Hence,

[1] *Ind. Eng. Chem. Anal. Ed.*, **5**, 7 (1933).
[2] *This Journal*, **16**, 105 (1933).

it is recommended in his report that the associate refereeship on this work be discontinued.

Several methods included in the chapter on plants have been adopted as official, first action, and others have been adopted as tentative. Although no collaborative work has been done on these for a few years, some of them are proving satisfactory in the hands of several chemists. These should be carried through to adoption, final action. Other methods have been deleted, first action. These should be carried to the final action. Hence, recommendations are made along these lines in this report.

<div align="center">RECOMMENDATIONS[1]</div>

It is recommended—

(1) That the method for the determination of fluorine referred to in this report be further studied with the purpose of making it applicable to plant materials and if feasible that collaborative work be done.

(2) That an associate referee be appointed to study methods for the determination of sodium in plants.

(3) That in *Methods of Analysis, A.O.A.C.*, 1930, 102, par. 5 and foot note, "10–50 g" be adopted as official, final action.

(4) That the method "Ferric and Aluminum Oxides—Official," and the expression in parentheses "Applicable to plant materials other than seeds" (*Methods of Analysis, A.O.A.C.*, 1930, 103, preceding par. 6) be deleted, final action.

(5) That the methods "Manganese, Calcium, and Magnesium—Official" and the expression in parentheses "Applicable to plant materials other than seeds" (*Methods of Analysis, A.O.A.C.*, 1930, 104, preceding par. 9), be deleted, final action.

(6) That the method "Calcium—Official, First Action" (*Methods of Analysis, A.O.A.C.*, 1930, 104, par. 9) be adopted as official, second action.

(7) That the micro method "Calcium—Tentative" (*Methods of Analysis, A.O.A.C.*, 1930, 105, pars. 10–12), be adopted as official, first action.

(8) That the method "Magnesium—Official, First Action" (*Methods of Analysis, A.O.A.C.*, 1930, 106, par. 13) be adopted as official, second action.

(9) That the magnesium nitrate method for the determination of Sulfur—Official, first action (*Methods of Analysis, A.O.A.C.*, 1930, 110, pars. 26–27) be adopted as official, second action.

(10) That Method I for the determination of phosphorus, official, first action (*Methods of Analysis, A.O.A.C.*, 1930, 110, par. 28) be adopted as official, second action.

(11) That the tentative micro method for the determination of phosphorus (*Methods of Analysis, A.O.A.C.*, 1930, 110, pars. 29–31) be adopted as official, first action.

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[1] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 48 (1933).

TABLE 1

Effect of storage in alcohol on the free reducing sugars and sucrose of plant tissues

TISSUE	FREE REDUCING SUGARS		DIFFERENCES		SUCROSE		DIFFERENCES	
	MARCH 26, 1931	MAY 25, 1932			MARCH 18, 1931	MAY 25, 1932		
	mg.	*mg.*	*mg.*	*per cent*	*mg.*	*mg.*	*mg.*	*per cent*
Cabbage leaves	676.0	665.0	−11.0	−1.6	68.5	70.7	+2.2	+3.2
Apple fruit	1110.0	1100.0	−10.0	−0.9	370.5	373.0	+2.5	+0.6
Carrots	495.0	496.0	+1.0	+0.2	414.0	418.0	+4.0	+0.9
Apple shoots	300.4	281.5	−18.4	−6.1	270.0	282.3	+12.3	+4.5
Tomato plants	209.0	207.2	−1.8	−0.8	79.0	83.6	+4.6	+5.8

REPORT ON PREPARATION OF PLANT
MATERIAL FOR ANALYSIS

By H. R. KRAYBILL (Purdue University Agricultural Experiment
Station, Lafayette, Ind.), *Associate Referee*

At the last meeting of this Association a recommendation was adopted[1] that the method of preparation of plant materials for analysis, adopted tentatively[2] in 1929, be adopted as official (first action) and that further studies be made with a view to its final adoption as official.

Samples of five plant materials—cabbage leaves, apple fruits, carrots, apple shoots, and tomato plants, which were used in the studies reported last year, were held in storage until May 25, 1932 and then analyzed. The results are given in Table 1 (p. 464).

In most cases the results show slightly smaller quantities of free reducing substances and larger quantities of sucrose on May 25, 1932, than on March 26, 1931. The differences are not greater than the usual errors of the methods used.

The results show that the method of preparation of samples by preserving in alcohol to which calcium carbonate has been added is satisfactory.

RECOMMENDATIONS[3]

It is recommended—

(1) That the method of preparation of plant materials for analysis, adopted tentatively in 1929 and as official (first action) in 1931, be adopted as official (final action).

(2) That the work on preparation of plant material for analysis be discontinued.

REPORT ON LESS COMMON METALS IN PLANTS

SPECTROSCOPIC METHOD FOR DETERMINATION OF
BORON IN PLANT MATERIAL

By J. S. McHARGUE (Kentucky Agricultural Experiment Station,
Lexington, Ky.), *Associate Referee*

A spectroscopic method for the determination of boron in plant material was worked out by R. K. Calfee, Department of Chemistry, Kentucky Agricultural Experiment Station, working under the supervision of the associate referee.

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[1] *This Journal*, 15, 45 (1932).
[2] *Ibid.*, 13, 62 (1930).
[3] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 48 (1933)

The method is as follows:

Weigh a portion of the plant material sufficient to contain 0.1 mg. of boron into a platinum or silica dish, saturate with a 10 per cent solution of boron-free potassium carbonate, evaporate to dryness, heat over a small flame until the volatile matter has been expelled, and then ash the residue in a muffle furnace at about 400° C. Cool, moisten the residue with distilled water, and add a saturated solution of citric acid until the carbonates are decomposed and the solution is distinctly acid. Transfer the solution to the reaction flask (C) (Fig. 1) with not more than 20 ml. of water, add 35 ml. of methanol, and distil for 1.5 hours. (Before the distillation put 0.1 ml. of 0.5 N potassium hydroxide solution and 10 ml. of distilled water into the receiver (F) (Fig. 1). After distillation, transfer the contents of the receiver, with a little water, to a small platinum or silica dish, add 5 ml. of ammonium hydroxide,
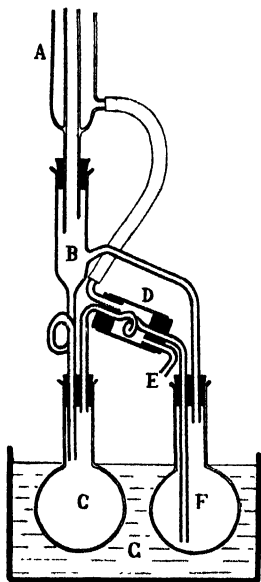
FIG. I.—DISTILLATION APPARATUS FOR METHYL BORATE: A–REFLUX CONDENSER, B–ADAPTER, C–DISTILLATION FLASK, D–VAPOR CONDENSER, E–WATER INLET, F–RECEIVING FLASK, G–WATER BATH
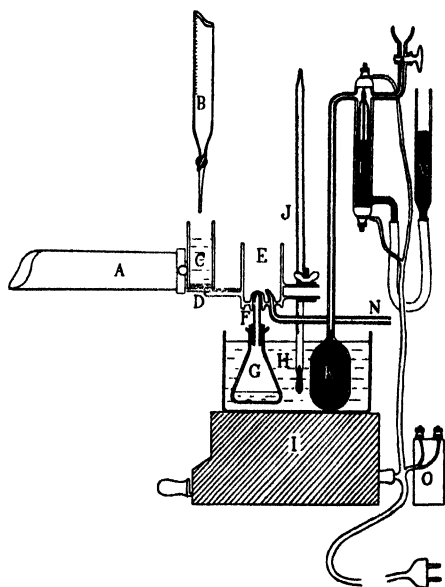
FIG. 2.—APPARATUS FOR THE SPECTROSCOPIC DETERMINATION OF BORON: A–SPECTROSCOPE, B–BURET, C–CELL, D–SUPPORT, E–CHIMNEY, F–BURNER, G–SAMPLE FLASK, H–BATH, I–HEATER, J–THERMOMETER, K–MERCURY BULB, L–THERMOSTAT M–REGULATION TUBE, N–PILOT LIGHT, O–CONDENSER

and evaporate to dryness. Dissolve the residue in a 5 per cent solution of phosphoric acid in methanol and make up to 25 ml. with more of the same solution, or a larger volume if much boron is present. Transfer 10 cc. of this solution to the Erlenmeyer flask (G) (Fig. 2), connect the burner (F), and when the flame of the methanol vapor has attained the prescribed conditions, observe the boron spectrum. Add
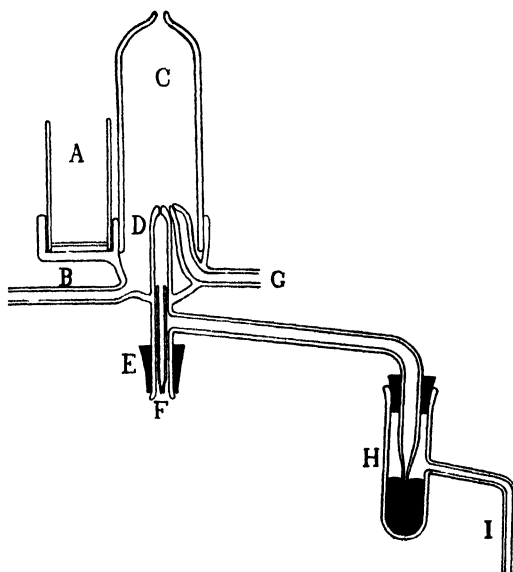
FIG. 3.—APPARATUS FOR BURNING METHYLBORATE IN OXYGEN: A–CELL, B–OXYGEN
INLET, C–CHIMNEY, D–MICRO BURNER, E–CONNECTION FOR FLASK,
F–VAPOR INLET (CAPILLARY), G–GAS INLET FOR PILOT LIGHT,
H–PRESSURE REGULATOR, I–ALCOHOL OVERFLOW
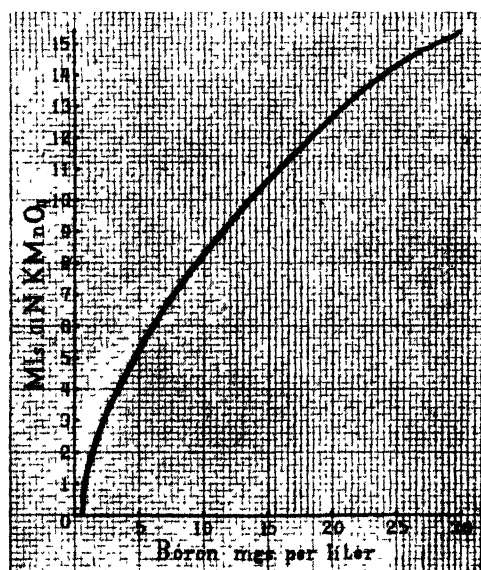
FIG 4.—AMOUNTS OF $0.01N$ KMnO₄ SOLUTION REQUIRED BY 50 ML. OF DISTILLED
WATER IN A 2 CM. CELL TO ELIMINATE THE FIRST GREEN BAND
FROM THE SPECTRA OF VARYING QUANTITIES OF
BORON BURNED IN OXYGEN

0.01 N potassium permanganate solution to the 50 ml. of water in the cell (C) (Fig. 2), until the brightest green band of the spectrum is just eliminated. Refer the quantity of potassium permanganate (ml.) required to a table that shows the corresponding weight of boron per liter in the solution in flask (G) (Fig. 2). This weight divided by the weight of substance taken gives the percentage of boron (grams B.

per liter $\dfrac{25}{1000} \times \dfrac{100}{\text{wt. taken}}$ $\Big\}$.

The table showing the grams per liter of boron present for any quantity of potassium permanganate solution used should be prepared by the operator from known quantities of boron, because the sensitivity of different spectroscopes and their adjustments vary widely. The reagents used must be free from boron.

TABLE 1

Synthetic ash (Salt mixtures)

	BORON (GRAMS)	
TREATMENT	ADDED	FOUND
1. MeOH left in contact with salt mixture for 18 hours at 28°C.	0.005	0.0046
	0.005	0.0044
		Av. 0.0045
	0.0075	0.0070
	0.0075	0.0072
		Av. 0.0071
2. Salt mixture extracted in distillation tube for ½ hour	0.0025	0.0012
	0.0025	0.0016
		Av. 0.0014
	0.0050	0.0030
	0.0050	0.0028
		Av. 0.0029
3. Salt mixture extracted in distillation tube for 1 hour	0.0025	0.0023
	0.0025	0.0023
		Av. 0.0023
	0.0050	0.0047
	0.0050	0.0048
		Av. 0.0048

TABLE 1 (*Continued*)

| TREATMENT | BORON (GRAMS) | |
	ADDED	FOUND
4. Salt mixture extracted in distillation tube for 1½ hours	0.0025	0.0025
	0.0025	0.0024
		Av. 0.0025
	0.0050	0.0050
	0.0050	0.0049
		Av. 0.0050
	0.0075	0.0076
	0.0075	0.0076
		Av. 0.0076
5. Salt mixture extracted in distillation tube for 2 hours	0.0025	0.0025
	0.0025	0.0025
		Av. 0.0025
	0.0050	0.0049
	0.0050	0.0050
		Av. 0.0050

TABLE 2

Water cress (25 gram samples)

| TREATMENT | BORON (GRAMS) | | |
	PRESENT	ADDED	FOUND
1. H_2O solution of ash evaporated and extracted with MeOH	—	—	0.0005
			0.0003
	—	—	Av. 0.0004
2. Citric acid solution of ash evaporated and extracted with MeOH	—	—	0.00035
			0.00036
	—	—	Av. 0.00036
3. Citric acid solution of ash evaporated with KOH and extracted with MeOH	—	—	0.0032
	—	—	0.0030
	—	—	0.0030
	—	—	0.0029
			Av. 0.0030

TABLE 2 (*Continued*)

TREATMENT	BORON (GRAMS)		
	PRESENT	ADDED	FOUND
4. Citric acid solution of ash extracted with MeOH by reflux and distillation	—	—	0.0030
	—	—	0.0030
	—	—	0.0031
			Av. 0.0030
5. Ditto	0.003	0.001	0.0040
	0.003	0.001	0.0038
	0.003	0.001	0.0039
			Av. 0.0039
6. Ditto	0.003	0.002	0.0049
	0.003	0.002	0.0052
	0.003	0.002	0.0051
	0.003	0.002	0.0050
	0.003	0.002	0.0052
			Av. 0.0051
7. Ditto	0.003	0.003	0.0058
	0.003	0.003	0.0058
	0.003	0.003	0.0059
	0.003	0.003	0.0060
			Av. 0.0059
8. Ditto	0.003	0.005	0.0081
	0.003	0.005	0.0078
			0.0079
			0.0079
			Av. 0.0079

The method is also applicable for the determination of boron in animal tissues and natural waters by making such modifications of the procedure as are necessary for obtaining the boron in suitable form for solution in methanol.

The method is sensitive to much smaller quantities of boron if the vapors of methanol methyl borate are burned in an atmosphere of oxygen. Fig. 3 shows the apparatus devised for burning the methylborate vapors in oxygen under controlled conditions. To insure the burning of the vapor at the same rate in each determination, constant pressure was

kept in the distillation flask by a mercury valve and capillary tube (H) (Fig. 3). Fig. 4 shows the grams per liter of boron in methanol represented by any quantity of 0.01 N potassium permanganate solution within the limits of the method. By burning in oxygen the method will detect the presence of less than 0.5 parts per million of boron in methanol. Quantities of boron between 3 and 30+parts per million can be determined quantitatively with an accuracy of ±0.35 parts per million.

The associate referee recommends[1] that the method for the spectroscopic determination of boron be made tentative and that co-operative work be carried on next year.

No report on total chlorine in plants was given by the associate referee.

REPORT ON CARBOHYDRATES IN PLANTS

By J. T. SULLIVAN[2] (Purdue University Agricultural Experiment
Station, Lafayette, Ind.), *Associate Referee*

The report presented at the 1931 meeting of the Association contained results of collaborative work on the method of clearing plant extracts with lead acetate, the determination of reducing sugars by the Quisumbing and Thomas method of reduction, and the gravimetric method for the determination of reduced copper. These methods were adopted as official. The collaborators also furnished data upon the sucrose content of the extracts studied. These data were lacking in uniformity. The procedure used in the determination of sucrose, furnished by T. G. Phillips,[3] is as follows:

Pipet 50 cc. of the cleared extract into a 400 cc. beaker. Add 2 drops of methyl red indicator and make slightly acid to methyl red by adding 1–2 drops of a 10 per cent acetic acid solution if necessary. Add 2–3 drops of a 1 per cent solution of Wallerstein's red label invertase. Cover with a watch-glass and let stand at room temperature for 2 hours. Add Fehling's solution and determine the reducing power.

The lack of uniformity in the results obtained indicates a fault in the procedure. While the method appears to be fairly accurate with small quantities of sucrose, some of the samples contained relatively large quantities.

The possible and more obvious errors may be tabulated as follows:

1.—Sucrose is determined by the difference between two reductions. The errors of each may be additive.

2.—Insufficient time for the action of invertase.

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[1] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 49 (1933).
[2] Presented by H. R. Kraybill.
[3] *J. Biol. Chem.*, 95, 735 (1932).

3.—Insufficient concentration of invertase.

4.—Unfavorable hydrogen-ion concentration.

5.—Changing of the concentration by evaporation of water upon standing or by the addition of the indicator, acetic acid, or enzyme solution.

The associate referee made some preliminary studies toward improving the procedure. Pure sucrose was used with the same concentration of enzyme as that mentioned previously. The solutions were kept standing in 100 cc. volumetric flasks instead of in beakers and were covered with toluene. After the solutions had been made to volume, 50 cc. was used for reduction. With quantities of sucrose as high as 100 mg., 2 hours' time appeared to be sufficient, as the reducing power at the end of that time was as great as that obtained on standing overnight. The presence of buffers to give a *p*H of 4.6, 5.2, and 6.0 did not influence the result. Smaller quantities of sucrose gave a maximum reducing power in a shorter time.

A number of plant extracts were subjected to the same treatment. One-half cc. of 0.5 per cent invertase solution was used. The results are given in the table.

Extract of Apple Fruit

(86.9 mg. of reducing sugar present)

NO BUFFER		ACID TO METHYL RED		PH 4.6		PH 5.2		PH 6.0	
hours	*mg.*	*hours*	*mg.*	*hours*	*mg.*	*hours*	*mg.*	*hours*	*mg.*
2:30	32.7	2:40	33.0	2:50	32.9	3:00	32.6	3:05	33.2
22:50	33.2	23:00	32.8	4:25	33.0	4:40	33.2	23:35	32.6
				24:20	32.6				

Extract of Cabbage

(67.2 mg. of reducing sugar present)

1:35	9.5	1:45	10.1	1:45	10.7	1:50	10.5	1:55	10.5
4:15	10.5	4:25	11.3	4:25	10.3	4:30	10.7	4:35	10.8
21:05	11.4	21:10	10.9	27:45	12.0	21:20	11.4	21:25	11.2

Extract of Carrots

(51.1 mg. of reducing sugar present)

1:05	28.1	1:00	44.0	1:50	37.4	1:40	45.1	1:55	37.4
2:00	37.4	25:00	45.4	25:00	45.6	25:05	45.7	25:10	45.6
24:50	45.2								

*Extract of Apple Twigs**

(30.5 mg. of reducing sugar present)

1:50	28.2			2:00	30.1	2:10	30.4	1:45	30.1
4:20	31.2			4:20	31.8	3:35	31.9	4:35	32.4
28:15	37.0			28:15	37.2	28:15	37.0	28:15	37.6
				47:50	38.2	47:50	37.4		

* Cleared extract was acid to methyl red.

Extract of Tomato Plants

(21.84 mg. of reducing sugar present)

2:20	8.86	2:25	8.39	2:15	8.95	2:25	9.15	2:25	9.15
4:05	8.77	4:05	8.86	4:10	9.33	4:15	8.30	23:15	9.24
16:10	9.15	16:10	9.15	16:15	9.06	23:10	9.06		
22:45	9.15	22:55	8.86	23:00	8.95				

Extract of Wheat Stems and Leaves

(31.2 mg. of reducing sugar present)

1:45	31.1	1:45	32.8	1:50	32.6	1:55	32.6	5:05	37.7
4:30	36.8	5:25	38.5	4:15	38.3	5:10	37.3	20:25	52 0
13:25	42.7	13:40	42.3	13:50	42.8	20:35	45.4		
20:20	50.7	20:10	50.9	20:05	52.2				

DISCUSSION

The results indicate that in some plant extracts it is not possible to obtain the maximum reducing power in two hours. With only one extract was the experiment continued longer than 24 hours, and there was still some increase beyond that. The hydrogen-ion concentration does not appear to play an important part in the final amount obtained. A higher concentration of invertase may hasten the hydrolysis, but experience has shown that larger quantities cause difficulty in the filtration of the reduced copper. It is apparent that a longer time than 2 hours is necessary. The question of the specificity of the invertase preparation cannot be raised on such meager data, but it may be significant that the extract of the wheat stems, which gave a persistent increase of reduction with time, has been shown to contain levulosans.[1]

RECOMMENDATIONS[2]

It is recommended—

(1) That the use of invertase in the determination of sucrose be studied further.

(2) That further studies be made upon methods for the determination of copper.

(3) That studies be made upon the determination of starch.

REPORT ON FORMS OF NITROGEN IN PLANTS

By HUBERT B. VICKERY[3] (Connecticut Agricultural Experiment Station, New Haven, Conn.), *Associate Referee*

Pursuant to the recommendation made in the previous report[4] further study was given to the problem of determining nitrate nitrogen in plant

[1] *Bull. soc. encour. ind. nat.*, No. 10, 605 (Oct. 1931).
[2] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 49 (1933).
[3] Presented by O. B. Winter.
[4] *This Journal*, 15, 516 (1932).

tissues. As a result of this study a method that depends on the extraction of nitric acid from the acidified tissue by means of ether was developed; the quantity is then determined by reduction to ammonia in essentially the same manner as is described in the report for 1930.[1] Collaborative study of this method will be made at the earliest opportunity.

NITRATE NITROGEN IN PLANT TISSUE

The method is fully described by Pucher, Vickery and Wakeman.[2] It was developed primarily for application to dried samples of tobacco, but it can be applied to other plant tissues or to extracts of plant tissues equally well.

REAGENTS

(a) *Sulfuric acid.*—4 N. Prepared from C.P. special reagent low in nitrogen.

(b) *Sulfuric acid.*—18 N. Prepared from C.P. special reagent low in nitrogen.

(c) *Sodium hydroxide.*—0.5N.

(d) *Concentrated sodium hydroxide.*[3]

(e) *Phenolphthalein indicator* (alcohol solution).

(f) *Reduced iron powder.*—Determine the titration value of the ammonia in the powder by boiling 3.0 grams of it with 50 cc. of 4 N sulfuric acid for 5 minutes, cool, make alkaline with sodium hydroxide, distil into 0.1 N acid, and titrate with 0.1 N alkali to methyl red. Divide by 10 to obtain the correction to be used with the 0.3 gram of powder employed in the method.

(g) *Ammonium sulfate stock solution.*—Dissolve 2.358 grams of pure salt in water and make up to 1000 cc.; 2 cc. = 1.0 mg. of nitrogen. Add no preservative.

(h) *Ammonium sulfate standard solution.*—Dilute 200 cc. of (g) to 1000 cc.; 1 cc. = 0.1 mg. of nitrogen.

(i) *Nessler's solution.*[4]

(j) *Hydrochloric acid.*—0.1 N.

(k) *Sodium hydroxide.*—0.1 N.

(l) *Methyl red indicator.*[5]

(m) *Asbestos fiber.*

(n) *Alcohol-free ether.*

(o) *Diphenylamine reagent.*—Suspend 0.5 gram of diphenylamine in 20 cc. of water and add concentrated sulfuric acid to 100 cc. Cool and preserve in a dark bottle.

DETERMINATION

Ascertain the quantity of 4 N sulfuric acid required to bring a 2.00 gram sample of the dried and powdered tissue to approximately pH 1.0 by weighing out 0.50 gram and stirring this in a small beaker with 1 cc. of 4 N acid; add enough water to make a thin paste that can be transferred to the electrode vessel, add quinhydrone, and determine the reaction at the potentiometer. Make suitable changes in the quantity of acid added to a second 0.5 gram sample, as suggested by the result of the first test, and repeat the determination. Continue until the quantity required to give a reaction in the range pH 0.7 to 0.9 has been found. Multiply this by 4 to obtain the amount required by the 2.00 gram sample used for the nitrate determination.

[1] *This Journal,* 14, 228 (1931).
[2] *J. Biol. Chem.,* 97, 605 (1932).
[3] *Methods of Analysis, A.O.A.C.,* 1930, 19 (i).
[4] *This Journal,* 14, 230 (1931).
[5] *Methods of Analysis, A.O.A.C.,* 1930, 19 (k).

Weigh duplicate 2.00 gram samples of the powder, mix each in a beaker with the required quantity of 4 N sulfuric acid until a uniform stiff paste is obtained; add 3.5 grams of pure asbestos fiber to each and incorporate thoroughly. Transfer the mixtures to 26×60 mm. Schleicher and Schüll paper extraction thimbles by means of a glass funnel about 11 cm. long, the upper part of which is a cylinder 4.5 cm. in diameter, the lower a cylinder 2 cm. in diameter. The transfer is accomplished as follows: Support the thimble in a wire cage hung in the mouth of a 400 cc. conical extraction flask and clamp the funnel in position over it so that the smaller end extends about 1 cm. into the thimble. Push most of the asbestos mixture into the thimble with a glass rod; brush off beaker, funnel, and rod; and wipe off all particles with a small piece of surgical cotton; finally rinse the glassware and brush into the thimble with alcohol-free ether. Remove the funnel and plug the end of the thimble with the cotton used to wipe the apparatus. Place the thimble in the siphon tube of the ether extraction apparatus (type designed for rubber analysis, Eimer and Amend catalogue No. 30754), thrust a short slim glass rod between the thimble and the glass in order to hold the paper away from the glass wall at one side, and suspend the siphon tube close under the metal coil condenser of the apparatus by means of a fine galvanized iron wire. Place 150 to 200 cc. of alcohol-free ether in the conical flask. Cut a gasket from soft cardboard to fit the recess in the plate of the metal condenser, set the condenser, with attached siphon tube, on the extraction flask and hold it firmly in position by means of three spring paper clips.

Place the extraction flasks on an electric hot plate, add a few angular quartz pebbles, and allow the extraction to proceed at least 8 hours at a siphoning rate of about 40 times per hour. If the rate is less a correspondingly longer time must be allowed. To test for the completeness of the removal of the nitric acid prepare a concentrated water extract of the residue in the thimble and overlay 5 cc. of the diphenylamine reagent in a test tube with a few cc. of this extract. The appearance of a blue layer at the junction of the two solutions indicates that the extraction of the nitric acid by the ether has been incomplete.

Treat each ether extract with 25 cc. of water, add 2 drops of phenolphthalein, and make faintly alkaline with 0.5 N sodium hydroxide with continual agitation. Immerse the flask in a water bath and evaporate off the ether very slowly to avoid frothing; make the aqueous solution to 100 cc. and transfer an aliquot part (10 cc. or more depending on the nitrate content of the tissue) to a 300 cc. Kjeldahl flask. Add 2.5 cc. of 18 N sulfuric acid and 0.3 gram of reduced iron powder. Boil gently for 5 minutes, cool, add 20 cc. of water and 10 cc. of ammonia-free concentrated sodium hydroxide. Immediately fit the flask with a Folin and Wright distillation tube[1] and distil over a micro burner for 5 minutes, counted from the time distillate first appears in the tube, into 3 cc. of 0.1 N hydrochloric acid contained in a test tube. Transfer the distillate to a 100 cc. flask, dilute to about 60 cc. with ammonia-free water, add 10 cc. of Nessler's solution, agitate, and make to volume. Prepare standard ammonia solutions by pipetting 3–15 cc. of the ammonium sulfate standard solution into 100 cc. flasks (0.3 to 1.5 mg. ammonia nitrogen), dilute, add 10 cc. of Nessler's solution, agitate, and make to volume. Read the color of the solution derived from the analysis in a colorimeter against nearest standard. Calculate the quantity of nitrogen in the aliquot part used for reduction, subtract the blank for the ammonia nitrogen found in the 0.3 gram of iron powder used, and calculate the nitrate nitrogen in the 2.00 gram sample taken. Express the final result as percentage of the dry tissue.

If the nitrate content of the tissue is of the order 0.1 per cent or less, it is desirable to carry out a blank determination on the alkaline solution of the ether extract. To do this proceed as follows.

[1] *This Journal,* **14,** 230 (1931).

Transfer an aliquot part of the alkaline solution equal to that used for the determination to a 300 cc. Kjeldahl flask, add 2.5 cc. of 18 *N* sulfuric acid, boil gently for 5 minutes, cool, add 20 cc. of water and 10 cc. of concentrated sodium hydroxide solution, and distil as already described. Transfer the distillate to a 25 cc. volumetric flask, dilute to 15 cc., add 2.5 cc. of Nessler's solution, agitate, and make to volume. Compare with ammonia standards of 0.05 to 0.10 mg. Deduct the quantity of ammonia nitrogen found from the quantity found after reduction with iron powder, correct the result for the blank due to the iron powder, and calculate the nitrate nitrogen as before.

To determine the nitrate content of extracts from plant tissue proceed as follows.

Transfer an aliquot part of the extract approximately equivalent to 2 grams of dry tissue to an evaporating dish, make neutral to Congo red if necessary, and evaporate to a sirup (it must *not* be evaporated to dryness). Cool and add the quantity of 4 *N* sulfuric acid, found by a separate experiment, to be required to produce a reaction in the range pH 0.7 to 0.9; add 3.5 grams of asbestos, and mix thoroughly. If the mixture is too moist to be transferred to the extraction thimble, dry it in a vacuum desiccator until this can be done. Proceed with the extraction as already described.

RECOMMENDATIONS[1]

It is recommended that collaborative study of this method for nitrate nitrogen be undertaken.

REPORT ON LIGNIN[2]

By MAX PHILLIPS (Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

At the last meeting of this Association a method for the quantitative estimation of lignin in plant materials was described.[3] Essentially the method consists in treating a weighed sample with fuming hydrochloric acid (d. 1.212–1.223 at 15°C.), which completely dissolves the cellulose and the other carbohydrates associated with the lignin, leaving the latter as an insoluble residue. From the weight of lignin obtained when corrected for ash and nitrogen the percentage of lignin in the sample can be calculated.

In order to determine the accuracy and reliability of the method in the hands of different analysts, the following experiments were performed.

Eight different types of lignified plant materials were selected for analysis. All the materials were ground to pass a 20-mesh sieve, dried in an oven at 105°C., extracted with an alcohol-benzene solution (32 parts by weight of 95 per cent ethanol and 68 parts by weight of benzene) for 6 hours in a large Soxhlet apparatus, again dried by suction, washed with hot water, dried at 105°C., and preserved in air-tight containers.

[1] For report of Subcommittee A and action of the Association, see *This Journal*, 16, 50 (193
[2] Contribution from the Color and Farm Waste Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.
[3] *This Journal*, 15, 124 (1932).

TABLE 1

Lignin in plant materials

(Results (except in the case of oat hulls) calculated on basis of original dry material. In the case of oat hulls the results are calculated on the basis of the extracted and dried material.)

PLANT MATERIAL	WEIGHT OF SAMPLE (ORIGINAL DRY MATERIAL)		EXTRACTED BY ALCOHOL-BENZENE AND BY HOT WATER	WEIGHT OF CRUDE LIGNIN (g)		N IN CRUDE LIGNIN	ASH IN CRUDE LIGNIN	WEIGHT OF LIGNIN CORRECTED FOR ASH AND PROTEIN		LIGNIN (CALCULATED ON ORIGINAL DRY MATERIAL)	
	(a)	(b)		(a)	(b)			(a)	(b)	(a)	(b)
	grams		per cent	gram		per cent	per cent	gram		per cent	
				Analyses made by Referee							
Corn Cobs	1.4052	1.4149	6.24	0.1676	0.1646	0.89	2.98	0.1534	0.1500	10.91	10.64
Wheat Straw	1.1088	1.1088	9.82	0.1760	0.1754	0.65	6.02	0.1584	0.1578	14.28	14.23
Spruce Wood	1.0495	1.1385	6.15	0.2903	0.3125	0.11	None	0.2885	0.3107	27.49	27.29
Wheat Bran	1.4317	1.3287	17.77	0.1195	0.1120	2.98	1.08	0.0963	0.0903	6.72	6.79
Timothy Hay	1.3829	1.3829	27.69	0.1748	0.1784	1.28	11.55	0.1408	0.1436	10.21	10.38
Corn Stalks	1.1418	1.1418	12.42	0.1938	0.1952	1.60	7.63	0.1598	0.1611	14.00	14.10
Oak Leaves	1.4045	1.0343	23.81	0.4223	0.4005	1.39	1.83	0.3780	0.3584	26.91	26.40
Oat Hulls	1.0248	0.9537	—	0.1724	0.1550	None	25.00	0.1293	0.1163	12.61	12.19
				Analyses made by R. W. Scharf							
Corn Cobs	1.1908	1.4950	6.24	0.1320	0.1666	0.96	3.33	0.1198	0.1511	10.06	10.10
Wheat Straw	1.3349	1.4165	9.82	0.2260	0.2384	0.81	7.97	0.1966	0.2074	14.73	14.64
Spruce Wood	1.5605	1.4162	6.15	0.4274	0.3862	0.18	0.10	0.4222	0.3815	27.06	26.94
Wheat Bran	1.4816	1.3666	17.77	0.1155	0.1072	2.99	0.26	0.0936	0.0869	6.32	6.36
Timothy Hay	1.7068	2.0611	27.69	0.2305	0.2815	1.73	14.28	0.1726	0.2107	10.11	10.22
Corn Stalks	1.2961	1.4195	12.42	0.2213	0.2398	0.88	9.59	0.1886	0.2036	14.55	14.33
Oak Leaves	2.0259	2.2906	23.81	0.6009	0.6740	0.82	1.07	0.5635	0.6320	27.81	27.59
Oat Hulls	1.3018	1.2733	—	0.2202	0.2153	0.33	26.98	0.1564	0.1529	12.01	12.01
				Analyses made by M. J. Goss							
Corn Cobs	1.0922	1.5159	6.24	0.1241	0.1774	1.06	6.25	0.1082	0.1547	9.91	10.20
Wheat Straw	1.8334	1.5881	9.82	0.2971	0.2566	0.63	5.66	0.2686	0.2320	14.65	14.60
Spruce Wood	1.4467	1.4339	6.15	0.3996	0.3954	0.10	0.10	0.3967	0.3926	27.42	27.38
Wheat Bran	2.2608	1.5039	17.77	0.1929	0.1253	3.02	0.88	0.1538	0.1006	6.80	6.68
Timothy Hay	1.4409	1.5238	27.67	0.1818	0.1947	1.19	9.90	0.1503	0.1610	10.43	10.56
Corn Stalks	1.2830	1.4464	12.42	0.2224	0.2456	1.26	6.79	0.1898	0.2097	14.79	14.49
Oak Leaves	1.4232	1.5466	23.81	0.4206	0.4619	1.34	1.19	0.3804	0.4178	26.73	27.01
Oat Hulls	1.0979	1.2592	—	0.1798	0.2066	0.33	27.03	0.1275	0.1465	11.61	11.63

To determine the loss in weight effected by the extraction process described, weighed samples (about 5 grams) of the oven-dried materials were similarly extracted with an alcohol-benzene solution and with hot water, and the percentage loss in weight was determined. This figure was then used in calculating the percentage of lignin in the original oven-dried unextracted material.

The lignin determinations were carried out exactly as described in the previous paper. The results obtained by the general referee and by the two collaborators are given in some detail in Table 1. The percentages of lignin are summarized in Table 2.

TABLE 2

Lignin in plant materials (Summary)

PLANT MATERIAL	(MEAN OF TWO DETERMINATIONS. RESULTS CALCULATED ON ORIGINAL DRY MATERIAL.)		
	REFEREE	E. W. SCHARF	M. J. GOSS
	per cent	er cpent	per cent
Corn Cobs	10.77	10.08	10.05
Wheat Straw	14.25	14.68	14.62
Spruce Wood	27.39	27.00	27.40
Wheat Bran	6.75	6.34	6.74
Timothy Hay	10.29	10.16	10.49
Corn Stalks	14.05	14.44	14.64
Oak Leaves	26.65	27.20	26.87
Oat Hulls*	12.40	12.01	11.62

* Results calculated on the dry extracted material.

It will be observed that the results obtained by the three analysts are of the same general order of magnitude, and considering the difficulties inherent in such an analytical method they may be said to be fairly good. It is only fair to state, however, that the method is not free from certain objectionable features, among which may be mentioned the unpleasantness connected with the handling and use of fuming hydrochloric acid. The acid cannot be kept for any length of time, for even at ice-box temperature it slowly gives off HCl and the residual acid accordingly becomes weaker. It is therefore necessary to prepare fresh batches of acid for each series of determinations. Furthermore, as will be observed from the tables, the lignin residue obtained is invariably contaminated with nitrogenous material. The method as now worked out calls for a determination of nitrogen (Kjeldahl) in the lignin residue, calculation of the crude protein from this value in the conventional manner, and deduction of this result from the weight of lignin obtained. In this procedure the assumption is made, of course, that the nitrogenous residue is in the nature of a protein. In view of the present knowledge of the chemistry of lignin it is perhaps the best procedure to follow, but it would be desir-

able to develop some method for the removal of the nitrogen-containing substances either before or after the plant material is subjected to the treatment with fuming hydrochloric acid.

In accuracy this method for the quantitative determination of lignin compares favorably with the known methods, but perhaps it would be desirable, before giving it an official status to do further investigational work in the direction of simplification and elimination of the objectionable features mentioned previously. It is therefore recommended[1] that further collaborative work be done on the quantitative estimation of lignin in plants and plant materials.

In conclusion the writer wishes to express his thanks to the collaborators, M. J. Goss, R. W. Scharf, and H. D. Weihe, for their helpful assistance and cooperation in making the various analyses assigned to them. The results obtained by Weihe are not included in this report, as unfortunately the analyses were carried out under different conditions.

REPORT ON ENZYMES*

By A. K. BALLS (Bureau of Chemistry and Soils,
Washington, D. C.), *Referee*

It was pointed out at the meeting last year that the only method of quantitative enzyme determination consists in a measurement of the enzymic effect itself. Therefore, while methods for several enzymes may resemble one another in principle, no two may be exactly alike. It is necessary to formulate a distinct procedure for each enzyme in which interest is centered.

As the consideration of analytical methods for enzymes constitutes (with a few exceptions) a new field of work for the Association, the referee believes it wiser not to undertake work on too many enzymes at once. Attention, therefore, has been paid to the determination of the enzyme catalase.

The selection of catalase was made for several reasons. Its importance as a factor in cell metabolism is rapidly being recognized, and a great amount of work on this ferment is now appearing. Differences in the catalase content of tissues under varying conditions of cell metabolism have been noted in the past by numerous authors. This field, however, is not only still unexhausted, but the underlying chemical reasons for these changes are as yet quite unknown. The apparent importance of catalase action as a part of tissue oxidation, and the presumable connection between this and cell proteolysis, make it seem probable that a great deal of interesting work on catalase may be expected in the future. Fur-

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thermore, many methods for catalase have been described, but experience in this laboratory has shown that they are open to various objections, not the least of which is the limited applicability of each scheme of determination.

It has been the aim of the referee to develop a method for catalase which would prove useful in determining this enzyme in raw materials, such as the products of agriculture, rather than exclusively in purified solutions, where the conditions are much simpler. It is too much to expect that the method proposed will serve for all sorts of agricultural products. It is hoped, however, that it will work with a rather wide variety of such materials, thus giving the advantage of comparing the catalase content of one material with that of another.

In pursuance of this object, the referee and W. S. Hale, also of the Food Research Division, have very carefully studied the most promising methods for catalase determination, and have developed a modification of the titrimetric method with which they are satisfied.[1]

It is now recommended[2] that this method be subjected to trial and criticism in various laboratories of the Association which are interested in the subject, with the object of ascertaining (1) how satisfactory the method is in the hands of other workers; (2) any modifications or improvement which might facilitate the analysis or considerably increase the accuracy of the determination; (3) the range of agricultural products with which satisfactory results may be obtained.

No report on dairy products was given by the referee.

No report on milk was given by the associate referee.

REPORT ON BUTTER

By C. W. HARRISON[3] (U. S. Food and Drug Administration, Minneapolis, Minn.), *Associate Referee*

The associate referee last year recommended[4] the adoption of the mechanical stirrer method as official and also that a study be made of the official method of preparing the sample with a view to clarifying some uncertainties which existed in the method. In lieu of this, Committee C suggested study of all promising methods of preparing samples of butter for analysis, with a view to finally adopting one or more methods which

[1] *This Journal*, **15**, 486 (1932).
[2] For report of Subcommittee A and action of the Association, see *This Journal*, **16**, 50 (1933).
[3] Presented by L. Hart.
[4] *This Journal*, **15**, 518 (1932).

appear most satisfactory. The proposal was too comprehensive to permit of its being carried out this year in its entirety; however, collaborative work was done to compare the official method of mixing the sample with a slightly modified mechanical stirrer method.

The contents of a small tub of butter were carefully melted in a large metal container, the temperature of the butter mass being kept between 31 and 34°C. while the butter was thoroughly mixed. While still fluid, sufficient subdivisions were poured into pint Mason jars to furnish each collaborator with two portions. The list of those who collaborated, all members of the U. S. Food and Drug Administration, follow:

1. R. S. Vandavere, Chicago   6. C. B. Stone, Minneapolis
2. R. S. Pruitt, Cincinnati   7. S. B. Falck, New Orleans
3. L. Jones, Kansas City   8. J. P. Aumer, St. Louis
4. A. W. Hanson, Minneapolis   9. L. Hart, Washington
5. S. M. Stark, Minneapolis

The collaborators were instructed to prepare one subdivision of the sample for analysis by following without any variation the official method[1] of mixing and to prepare the other subsample, using the following modification of the mechanical stirrer method:

Place the jar in a bath of water and gradually raise the temperature of the water to not exceeding 35° C. and maintain it at this temperature until the butter is thoroughly softened and the temperature of the butter mass is between 31 and 34° C. Mix thoroughly with a mechanical stirrer of the malted milk type. Complete the operation by thoroughly incorporating and mixing into the butter mass, with a spatula, any butter adhering around the edges of the jar. Immediately on completing the respective mixing proceed with the analysis of the two subsamples.

The collaborators were also requested to determine moisture by the official method;[2] residue by the official method, fat by official indirect method (1), and salt as follows: The crucible containing the dry residue after completion of salt and curd determination is treated with several portions of water and the washing continued until the salt is washed completely from the curd. Salt is determined by the volumetric method.

The analysis of the subsamples mixed by the two procedures is given in the following table:

TABLE 1

Collaborative results by the official and mechanical stirrer methods
(Results expressed in percentage)

	"A" OFFICIAL METHOD				"B" STIRRER METHOD (MODIFIED)			
	MOISTURE	RESIDUE	SALT	FAT	MOISTURE	RESIDUE	SALT	FAT
(1)	16.31	3.34	2.45	80.35	16.32	3.40	2.43	80 28
	16.31	3.38	2.46	80.31	16.28	3.36	2.44	80.36
Av.	16.31	3.36	2.46	80.33	16.30	3.38	2.44	80.32

[1] *Methods of Analysis, A.O.A.C.*, 1930, 231.
[2] *Ibid.*, 236.

	"A" OFFICIAL METHOD				"B" STIRRER METHOD MODIFIED			
	MOISTURE	RESIDUE	SALT	FAT	MOISTURE	RESIDUE	SALT	FAT
(2)	16.35	3.58	2.48	80.07	16.35	3.58	2.45	80.07
	16.35	3.65	2.49	80.00	16.35	3.51	2.46	80.14
	16.40	3.59	2.51	80.01	16.34	3.57	2.45	80.08
					16.40	3.60	2.47	80.00
Av.	16.37	3.60	2.49	80.03				
					16.36	3.57	2.46	80.07
(3)	16.46	3.42	2.50	80.12	16.31	3.45	2.42	80.24
	16.43	3.38	2.49	80.19	16.30	3.41	2.50	80.29
	16.42				16.26			
Av.	16.44	3.40	2.50	80.16	16.29	3.43	2.46	80.27
(4)	16.32	3.56	2.49	80.12	16.27	3.51	2.43	80.22
	16.30	3.39	2.31	80.31	16.31	3.51	2.43	80.18
	16.26	3.50	2.46	80.24	16.35	3.59	2.49	80.06
Av.	16.29	3.48	2.42	80.22	16.31	3.54	2.45	80.15
(5)	16.29	3.38	2.43	80.33	16.25	3.33	2.38	80.42
	16.25	3.39		80.36	16.20	3.33	2.38	80.47
	16.28	3.38	2.41	80.34	16.21	3.34	2.40	80.45
Av.	16.27	3.38	2.42	80.34	16.22	3.33	2.39	80.45
(6)	16.27	3.42	2.41	80.31	16.25	3.42	2.43	80.33
	16.29	3.44	2.43	80.27	16.28	3.40	2.40	80.32
	16.30	3.37	2.40	80.33	16.29	3.45	2.43	80.26
Av.	16.29	3.41	2.41	80.30	16.27	3.42	2.42	80.30
(7)	16.26	3.42	2.37	80.32	16.24	3.38	2.41	80.38
	16.25	3.44	2.43	80.31	16.28	3.40	2.35	80.32
Av.	16.26	3.43	2.40	80.32	16.26	3.39	2.38	80.35
(8)	16.26	3.43	2.47	80.31	16.18	3.49	2.45	80.33
	16.24	3.40	2.42	80.36	16.17	3.50	2.48	80.33
	16.17	3.35	2.45	80.48	16.16	3.50	2.46	80.34
Av.	16.22	3.39	2.45	80.39	16.17	3.50	2.46	80.33
(9)	16.28	3.39	2.37	80.33	16.24	3.40	2.41	80.36
	16.26	3.36	2.33	80.38	16.26	3.42	2.38	80.32
	16.36	3.31	2.35	80.33	16.26	3.42	2.40	80.32
					16.24	3.40	2.37	80.36
Av.	16.30	3.35	2.35	80.35				
					16.25	3.41	2.39	80.34

The results show that both methods of mixing were satisfactory and produced a homogeneous mass, nor was there any indicated preference between the two mixing methods as shown in Table 2.

TABLE 2

Compilation of average results

Official Method

MOISTURE per cent	RESIDUE per cent	SALT per cent	FAT per cent
16.31 (25)*	3.43 (24)	2.43 (23)	80.27(24)

Stirrer Method

| 16.27 (27) | 3.45 (26) | 2.43 (26) | 80.28 (26) |

Maximum Variations between Individual Averages

	OFFICIAL per cent	STIRRER per cent
Moisture	0.22	0.19
Residue	0.25	0.24
Fat	0.36	0.38

* Number of determinations.

It is concluded from these results that either the official method or the slightly modified stirrer method, as given here, is a satisfactory procedure for mixing butter samples for analysis.

Last year[1] it was recommended and approved (second action) that the procedure for sampling tub and print butter proposed in the previous year's report[2] be adopted as official. It is now believed that further action in adoption of this method as official should be deferred to permit of further study of another phase of the sampling operation.

The decision has been reached that under the terms of the Food and Drugs Act, each individual package in an interstate shipment is a unit and if deficient in fat a violation of this law. Therefore, a legal sample in the enforcement of that law should represent not a composite of the shipment, but so far as possible the unit package in the lot.

The methods of this Association having a semi-legal status[3] under the Food and Drugs Act, it seems advisable to have the methods, so far as possible, applicable under the enforcement regulations for the statute and to that end the sampling procedure should be extended to include a sampling procedure applicable to the individual unit in a given lot, and the present procedure for sampling should also cover cubes as well as prints and tubs of butter.

Another topic which is pressing for attention is the development of methods for the detection of sweet cream butter. This product is becoming more and more an article of commerce and it is probable that much of the material now being offered to consumers as "Sweet Cream Butter" is not in fact entitled to this designation. Methods should be developed as soon as possible for differentiating the genuine from the spurious article

[1] *This Journal*, 15, 57 (1932).
[2] *Ibid.*, 14, 232 (1931); *Methods of Analysis, A.O.A.C.*, 1930, 231.
[3] S. R. A. No. 1, p. 4.

RECOMMENDATIONS[1]

It is recommended—

(1) That the procedure for sampling tub, cube, and print butter be further studied.

(2) That the associate referee study the composition of the individual units in the churning with a view to recommending a suitable sampling procedure to cover the unit package as well as the average unit in the churning or batch.

(3) That the modified stirrer method outlined in this report receive further collaborative study.

(4) That the methods for determining salt and curd receive further study by the associate referee, especially with a view to determining the best method of removing the last traces of fat.

(5) That the associate referee inaugurate some work looking to the development of methods for distinguishing sweet cream butter from neutralized butter, with special reference to detection of neutralizers and starters.

REPORT ON CHEESE
LACTOSE AND SUCROSE IN PROCESS CHEESE

By CARL B. STONE (U. S. Food and Drug Administration, Minneapolis, Minn.), *Associate Referee*

At the 1931 meeting Subcommittee C recommended that methods for the determination of lactose and sucrose in cheese be further studied.[2]

A method presented by E. O. Huebner[3] was selected for study. The few changes made were first tested by the associate referee and were then included in the directions sent to the collaborators. These changes have been published.[4]

A batch of cheese was prepared in a plant that manufactures process cheese. The ingredients used provided for 4.70 per cent lactose and 0.54 per cent sucrose in the finished cheese, but owing to the possibility of some loss of moisture in manufacture, the actual percentage present in the finished product might be somewhat in excess of the theoretical values.

The sample for the collaborators was mixed thoroughly by being passed through a meat chopper and was forwarded in a suitable container under dry ice refrigeration to the different analysts, who were requested to determine moisture, lactose, and sucrose on the prepared sample. Their results are given in Table 1.

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 60 (1933).
[2] *This Journal*, 15, 58 (1932).
[3] *Ibid.*, 13, 243 (1930).
[4] *Ibid.*, 16, 72 (1933).

TABLE 1

Collaborative results on lactose and sucrose in process cheese.

COLLABORATORS	LACTOSE	SUCROSE	TOTAL SUGARS RECOVERED AS LACTOSE AND SUCROSE
	per cent	*per cent*	*per cent*
F. J. McNall	4.95	0.37	5.34
U. S. Food and Drug Adm. Chicago	4.96	0.38	
F. A. Vorhes, Jr. U. S. Food and Drug Adm. Washington	3.91*	0.79*	4.70*
Samuel Alfend	4.73	0.44	5.17
U. S. Food and Drug Adm. St. Louis	4.71	0.45	
C. W. Elmer Swift & Co. St. Paul	4.88†	0.20†	5.18†
Geo. A. Dysterheft Agr. Dairy & Foods St. Paul	4.81	0.38	5.19
J. L. Perlman	4.85	0.38	5.23
Agr. & Markets	4.83	0.38	
Albany	4.87	0.38	
Guy G. Frary	4.99	0.37	5 35
Vermilion, S.D.	4.98	0.35	
	5.02	0.34	
S. M. Stark, Jr.	4.92	0.33	5.25
U. S. Food and Drug Adm.	4.88	0.32	
Minneapolis	4.97	0.33	
Carl B. Stone	4.96	0.30	5.26
	4.97	0.30	
	4.91	0.30	
Maximum	5.02	0.45	5.35
Minimum	4.71	0.30	5.17
Average	4.90	0.36	5.26

* Not included in the average as Mr. Vorhes stated the cheese was in poor condition when received, and the results reported confirm his statement.

† Not included in the average as this was a sample taken by Mr. Elmer from the original batch but not mixed by the associate referee. His figures represent an average of five analyses.

As a further study of the method the associate referee also prepared three additional cheese mixtures. The first mixture consisted of whole dried milk and skim milk powder designed to furnish a finished cheese containing 2.76 per cent of lactose. This is designated as Sample 2. The next batch was prepared by the addition of skim milk powder to the cheese mixture to furnish a process cheese containing 3.43 per cent of lactose and is designated as Sample 3. An additional batch was prepared by adding whole milk powder to the cheese mixture to furnish a process cheese containing 2.5 per cent of lactose. It is designated as Sample 4. The samples from these three batches were ground and thoroughly mixed and then submitted to analysis. The results are reported in Table 2.

TABLE 2

Results on Samples 2, 3 and 4.

ANALYST	SUB.	REDUCING SUBSTANCES BEFORE INVERSION AS LACTOSE	REDUCING SUBSTANCES AFTER INVERSION AS LACTOSE
		per cent	*per cent*
Sample 2			
S. M. Stark, Jr.	A	2.71	2.88
		2.72	2.96
C. B. Stone	B	2.55	2.67
		2.70	2.75
	Average A and B	2.67	2.82
Sample 3			
S. M. Stark, Jr.	A	3.08	3.18
		3.10	3.26
C. B. Stone	B	3.13	3.07
		3.17	3.13
	Average A and B	3.12	3.16
Sample 4			
S. M. Stark, Jr.	A	2.49	2.73
		2.54	2.78
C. B. Stone	B	2.46	2.59
		2.51	2.52
	Average A and B	2.50	2.66

Table 1 shows that several analysts working on several subdivisions of the same cheese sample containing lactose and sucrose secured very close results. The lactose figures are high, but this is because a certain amount of sucrose is inverted during the process of manufacture. On this account

the sucrose figure is low and a larger variation in check results is noted. The theoretical figure of 4.70 per cent would be somewhat higher owing to possible moisture lost so that the average of the seventeen analyses (4.90 per cent) is in reality a trifle closer than the figures indicate. Another factor which would lower the lactose average is that all the reducing substances before inversion were calculated as lactose, when in reality a certain amount of reducing material is invert sugar. If this inverted sucrose could be accurately calculated in each case the lactose would yield a lower figure, approximately 0.107 per cent. The total sugars recovered calculated as lactose and sucrose yield an average of 5.26 per cent, against the theoretical of 5.24 per cent. This indicates that the method will recover the added lactose and sucrose.

The results shown in Table 2 give a fairly good idea of how much lactose can be recovered when added in the form of milk powders. No reason can be given for the particular discrepancy shown with Sample 3.

The comments by the different collaborators indicate that the method is satisfactory. The results reported confirm this, as the total recovery of added reducing substances to a cheese mixture is very close to the theoretical. The one word of warning is that the reduction before inversion should be completed promptly owing to the acid present in the solution.

RECOMMENDATIONS

It is recommended[1]—

(1) That the method developed by Huebner for determining lactose and sucrose in cheese, with changes in wording suggested herein to bring it into conformity with the *Methods of Analysis, A.O.A.C.*, 1930, be made tentative.

(2) That further work be done on methods for the detection and estimation of added preservatives or other ingredients as gums, etc.

(3) That further work on the phosphorus pentoxide-calcium oxide ratio on process cheese be dropped.

No report on dried milk was given as no associate referee had been appointed.

REPORT ON MALTED MILK

By Fred Hillig (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The Food and Drug Administration of the Department of Agriculture developed a method for the determination of citric acid in milk, and the

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[1] For report of Subcommittee C and action of the Association. *see This Journal*, 16, 61 (1933).

several districts of this Administration were requested to analyze market milks for their citric acid content by this method. The reports received indicate that the citric acid content of milk is almost a constant (0.16 per cent).

With this information, the associate referee hoped to be able to approximate the milk solids and butter fat in a malted milk by a citric acid determination. This should also indicate whether or not a foreign fat had been added in the processing of the product. However, owing to a shortage of time, the work did not progress to a point where anything definite can be reported. It is believed, however, that the idea has possibilities.

It is also the intention to study during the coming year the determination of casein in malted milk and in the chocolate-flavored products appearing on the market, with a view to estimating the milk solid content by such a determination.

Time did not permit a study of the determination of the total amount of butter fat in a malted milk.

It is recommended[1]—

(1) That the determination of the Reichert-Meissl number of the fat of malted milk by the use of the method of extraction reported by the associate referee be studied collaboratively and that he also study the determination of the total amount of butter fat in a malted milk.

(2) That a method for the determination of citric acid in malted milk be studied.

(3) That a method for the determination of casein in malted milk be studied.

REPORT ON ICE CREAM

By G. G. FRARY (State Chemist, Vermilion, S. D.), *Associate Referee*

Following the recommendation of last year concerning study of modifications of the Babcock method which might be applicable to ice cream, the associate referee carried out some preliminary trials. The results of these trials are not encouraging.

Routine inspection samples were used, and the fat was determined by the official method and by one or more of three modifications of the Babcock method, depending upon the quantity of samples available. The three non-official methods tried include one which has been used for several years in the laboratory of the referee, employing acetic and sulfuric acids; a non-acid method developed at the University of Illinois Agricultural Experiment Station in 1930 (Bull. 360); and a method de-

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 60 (1933).

veloped in the same year at the University of Nebraska Experiment Station (Bull. 246).

The Illinois method employs two reagents, one of which is a mixture of ammonium hydroxide, normal butyl alcohol and ethyl alcohol, while the other is a concentrated solution of trisodium phosphate and sodium acetate in water. Using these reagents, the Illinois experimenters obtained fairly satisfactory results on a large number of samples of varying character. Approximately 89 per cent of the samples gave results differing not more than 0.2 per cent from the results obtained by the official Roese-Gottlieb method.

In the study with the Nebraska method approximately 80 per cent of the samples examined checked within 0.2 per cent of the results obtained by the official method and the use of the Mojonnier apparatus. Here the reagents are two, of which the first is a mixture of one part of strong ammonium hydroxide with nine parts normal butyl alcohol, and the second a mixture of equal volumes of sulfuric acid and ethyl alcohol. While results in the writer's laboratory showed much less favorable agreement than the results obtained by the official method, this fact may be due to the lack of familiarity with the newer methods.

It appears from a cursory survey of the practices in several States that modified Babcock procedures are in rather general use in regulatory laboratories. It is desirable that such methods be uniform.

It is, therefore, recommended[1] that the Nebraska and Illinois methods for the determination of fat in ice cream by modified Babcock procedures be studied collaboratively.

REPORT ON MILK PROTEINS

By Marie L. Offutt[2] (U. S. Food and Drug Administration,
New York, N. Y.), *Associate Referee*

The previous Associate Referee on Milk Proteins recommended[3] that the method he had worked out be given collaborative trial with a view to its adoption as official. No work was done on this subject last year, but this year the method was submitted to collaborators. The results are given in Table 1. Each collaborator obtained his own samples.

The collaborators were also requested to determine the albumin in the same samples by the official method (Method I) *Methods of Analysis, A.O.A.C.*, 1930, 215 and by the following proposed method:

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 61 (1933).
[2] Presented by L. Hart.
[3] *This Journal*, 14, 246 (1931); *Methods of Analysis, A.O.A C.*, 1930, 215

Pipet 250 cc. of normal acetic acid into a 1000 cc. flask. Add 125 cc. of normal carbon dioxide-free sodium hydroxide. Make up to 1000 cc. with carbon dioxide-free distilled water and mix thoroughly.

DETERMINATION

Pipet 20 cc. of the sample into a 100 cc. flask. Add 50 cc. of reagent, mix, make up to volume with distilled water, and shake well. Set the flask in hot water (50–60° C., not over 60°), and let stand 15 minutes. Cool to room temperature, add 0.5 gram of filter-cel, shake thoroughly, and filter clear through a suitable folded paper, taking care to prevent evaporation during filtration. Determine nitrogen (A) in 50 cc. of clear filtrate. Pipet another 20 cc. of sample into a 100 cc. flask and remove casein as above; then neutralize entire filtrate (A) thus obtained with 10 per cent sodium hydroxide solution, add 0.3 cc. of acetic acid (1+1), and heat on a steam bath until the albumin is completely precipitated. Filter quickly and determine nitrogen (C) in 50 cc. of this filtrate. Multiply (A–C) by 6.38. This gives the albumin in 10 cc. of the milk. Report grams of albumin per 100 cc. of milk.

TABLE 1

Results of determinations of casein in milk

(Expressed as grams per 100 cc.)

COLLABORATOR	SAMPLE	TOTAL PROTEIN "A" (N×6.38)	CASEIN, OFFICIAL METHOD	CASEIN (A–B), TENTATIVE METHOD	DIFFERENCE
Marie L. Offutt	1	3.22	2.40	2.52	+0.12
	2	3.20	2.54	2.54	0.00
	3	3.26	2.53	2.41	−0.12
	4	3.18	2.42	2.35	−0.07
	5	3.21	2.47	2.51	+0.04
J. T. Keister	1	3.24	2.55	2.66	+0.09
U. S. Food and			2.56 av. 2.56	2.63 av. 2.65	
Drug Adm.	2	3.16	2.37	2.45	+0.08
Washington			2.35 av. 2.36	2.43 av. 2.44	
T. B. Benjamin	1	3.28	2.47	2.48	+0.01
U. S. Food and	2	3.27	2.47	2.49	+0.02
Drug Adm.					
Chicago					

The results by this method and by the official method are given in Table 2.

Both collaborators suggested that in precipitating the albumin by heating on a steam bath the volume of filtrate might be less than 50 cc. and that it might be advisable to make up to a definite volume before removing an aliquot. In view of these criticisms and of the results ob-

TABLE 2

Results of determinations of albumin in milk

(Expressed as grams per 100 cc.)

COLLABORATOR	SAMPLE	OFFICIAL METHOD	PROPOSED METHOD	DIFFERENCE
Marie L. Offutt	1	0.32	0.25	−0.07
	2	0.36	0.41	+0.05
	3	0.27	0.31	+0.04
	4	0.39	0.35	−0.04
J. T. Keister	1	0.26	0.39	+0.12
		0.27 av. 0.27	0.39 av. 0.39	
	2	0.40	0.32	
		0.42 av. 0.41	0.33 av. 0.33	−0.08
T. B. Benjamin	1	0.49	0.35	−0.14
	2	0.49	0.35	−0.14

tained it seems advisable to change the proposed method for albumin to read as follows:

REVISED PROPOSED METHOD

DETERMINATION

Pipet 20 cc. of the sample into a 100 cc. flask. Add 50 cc. of reagent, mix, make up to volume with distilled water, and shake well. Set the flask in hot water (50–60° C., not over 60°) and let stand 15 minutes. Cool to room temperature, add 0.5 gram of filter-cel, shake thoroughly, and filter clear through a suitable folded paper, taking care to prevent evaporation during filtration. Determine nitrogen (A) in 50 cc. of clear filtrate. Pipet another 20 cc. of sample into a 100 cc. flask and remove casein as directed above, then place 50 cc. of this filtrate (A) thus obtained in a 100 cc. flask, neutralize with 10 per cent sodium hydroxide solution, add 0.3 cc. of acetic acid (1+1), and make up to 100 cc. Heat on steam bath in this flask until the albumin is completely precipitated. Filter quickly and determine nitrogen (C) in 50 cc. of this filtrate, and multiply this result by 2 before subtracting from A. A–C multiplied by 6.38 gives the albumin in 10 cc. of the milk. Report grams of albumin per 100 cc. of milk.

RECOMMENDATIONS

It is recommended—

(1) That the proposed method for the determination of casein be continued as tentative and be further studied.

(2) That the revised proposed method for the determination of albumin be further studied.

(3) That a method for the determination of casein in milk powders be studied.

No report on naval stores was given by the referee.

REPORT ON TURPENTINE

By F. P. VEITCH (Bureau of Chemistry and Soils,
Washington, D. C.), *Referee*

The Association's methods for the analysis of spirits of turpentine are the best known. No modification of the present methods or new methods worthy of serious consideration have come to the attention of the Referee on Turpentine during the year, consequently no cooperative work was necessary.

It has been found impracticable to do any work on methods for the examination of rosin during the past year, and there has been no offer of cooperation for active work on these methods. This condition is probably due to the pressure of what seemed to be more important work and an inadequate staff. Some work has been initiated, however, and it is hoped that during the next three or four months something worth while can be accomplished. There is real need for uniform methods for the analysis of rosin. The results by different procedures are rarely in agreement and analysts are, as a rule, not familiar with rosin.

No report on paints, paint materials, and varnishes was given by the referee.

REPORT ON CEREAL FOODS

By J. A. LECLERC (Bureau of Chemistry and Soils,
Washington, D.C.), *Referee*

For the second time in the history of the A.O.A.C., which is today holding its 48th annual meeting, the cereal chemists are assembled under conditions making possible more intimate contact and more thorough discussion of the problems of special interest.

Cereal chemists are interested in all phases of investigation pertaining to cereals: milling, baking, the manufacture of the various cereal products, the influence or effect of environment upon the nature and quality of the cereals themselves, the chemical and other changes which take place in the various processes such as milling, baking, malting, and in the manufacture of cereal foods in general. Cereal crops account for nearly one-half of the value of all farm crops produced in this country. Cereal foods constitute fully 25 per cent of the value of all manufactured food products. There are only four billion-dollar food industries in this country, and milling and baking comprise two of them.

Cereal foods supply more than one-fourth of the protein and over one-third of the energy of all foods ingested by man. The average per capita

consumption of flour is 175 pounds or the equivalent of a little over four bushels of wheat. Of the 110 million barrels of flour consumed in this country, probably 75 millions are for bread, 5 millions for biscuits and crackers, 3.5 millions for macaroni, besides 10 millions for self-rising and phosphated flours, the remainder being used for pies, pretzels, doughnuts, cakes, and sweet goods of all kinds. In the utilization of these 110 millions of barrels of flour and approximately $800,000,000 worth of food materials other than flour are required, two-thirds of which are products of the farm.

The cereal chemist is therefore in a position to play a most important role in the economic development of this country, and his profession gives him a splendid opportunity to be of service to his fellow man.

Whatever progress is accomplished as the result of these studies is largely due to the work of the associate referees and their collaborators, and to them the referee gladly gives credit.

L. H. Bailey and his collaborators showed that closely concordant results in moisture determinations can be obtained by either the vacuum method or the 130° air-oven method with bread, cake, crackers, pretzels, etc.

R. J. Clark reported that five chemists, working with a patent and a clear flour obtained remarkably close pH readings by both the electrometric and the colorimetric methods.

D. B. Scott, working with J. L. Hogan on the chlorine determination in flour, obtained encouraging results by using a modification of the Seidenberg method. Satisfactory progress was also made on the subject of bleaching of flour by the use of benzoyl peroxide.

L. C. Mitchell made a serious study of the method for the determination of fat in flour, macaroni, and baked products by acid hydrolysis and found that the temperature of extraction is very important. This associate referee also studied the applicability to flour, macaroni, and baked products of the method for determining phosphorus in eggs and found that when the quantity of Na_2CO_3 recommended for eggs is doubled and the temperature raised from 500° to 700°C., this method can be used for the various cereal products mentioned. A limited amount of study was also made to improve the present tentative (official, 1st action) method for the determination of lipoids in flour, baked goods, and noodles by extracting the lipoids from the wetted sample without previous removal of the water and the method for the determination of the water-soluble nitrogen precipitable by 40 per cent alcohol.

J. H. Bornmann made an important digest of the methods (mostly foreign) proposed for the detection of rye flour in wheat flour.

M. J. Blish secured the collaboration of eight chemists in his study of the diastatic value of flour. It was definitely established that the quantity of reducing sugars originally present in ordinary flour is actually so small

and so nearly constant as to be negligible, thus making it evident that the so-called "blank" determination may be dispensed with altogether.

R. G. Capen conducted collaborative studies on the determination of crude fiber in macaroni products, bread, and cake. Very good results were obtained with both bread and macaroni and encouraging results with cake.

L. Jones, ably assisted by the special studies of Munsey and by a dozen collaborators, reported that the modified Rask method for starch determination in flour (both patent and whole wheat) gives remarkably good results.

C. G. Harrel made certain recommendations (not however based upon collaborative work) regarding the tentative baking test. With slight modifications they are a general approval of the recommendation adopted by the A.A.C.C., as a result of the activities carried on under the provision of the Research Fellowship.

L. D. Whiting studied the method for the determination of CO_2 in self-rising flour, but no collaborative work was done.

A. Johnson studied methods for the determination of milk solids in bread by three methods, viz: (1) the loss obtained by drying air-dried bread at 130°; (2) the amount of casein in bread as determined by the aid of pyridine; (3) the amount of lactose in the crumb of the bread. The third method was found to be worthy of further trial.

Because of unavoidable circumstances, S. Alfend was unable to conduct work on the unsaponifiable matter in flour, bread, and alimentary paste. The same may be said of D. A. Coleman with regard to the color of flour and the method of determining ash in baked products.

No report was made by C. H. Bailey on foreign methods of testing flours. In this connection it may be of interest to know that at the first International Congress of Panification held in Rome and Bologna, Italy, the past summer, resolutions were adopted calling attention to the necessity of having international standard methods of analysis applicable to wheat flour, bread, etc., for the benefit of agriculture, the milling-baking industries and the consuming public. A special committee was appointed to consider this matter. Such action on the part of the Rome Congress should certainly stimulate this Association to study the methods which have been adopted by the various foreign governments for the analysis of cereals and cereal products. Foreign chemists interested in these problems will soon be found among our collaborators.

The recommendations submitted by the referee were published in the report of Subcommittee C, *This Journal*, 16, 62 (1933).

The paper "Tailor-made Flour" presented by F. L. Dunlap was published elsewhere.[1]

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[1] Baker's Helper, April 8, 1933, p. 508.

No report on ash in flour and in alimentary paste, ash and chlorine in baked products and color in flour was given by the associate referee.

REPORT ON H-ION CONCENTRATION OF FLOUR

By ROWLAND J. CLARK (436 W. Dartmounth Road, Kansas City, Mo.), *Associate Referee*

The purpose of the work described in this report was to devise a method by which the H-ion concentration of flour could be determined as quickly and as accurately as possible, with special attention given to the colorimetric method of procedure. Inasmuch as a method has been worked out for the electrometric determination of H-ion concentration, it was believed advisable to follow this method as far as possible for the colorimetric determination, thereby simplifying the procedures as much as possible and gaining the advantage of the data supporting the electrometric method. With this purpose in mind the procedure below was recommended for collaborative study. This method differs only in the last sentence from the electrometric method given in *Methods of Analysis, A.O.A.C.*, 1930, chap. XX, sec. 11. The method used for the colorimetric determination of H-ion concentration is as follows:

Weigh 10 grams of flour (or some multiple thereof) into a clean, dry Erlenmeyer flask and add for each 10 grams of flour 100 cc. of distilled water at a temperature of 25°C. Shake or whirl the flask until the particles of flour are evenly suspended and the mixture is free from lumps, place in a thermostat at 25°, and shake, continuously or intermittently in such a manner as to keep the flour particles in suspension, for 30 minutes.

Let stand quietly for 10 minutes, then decant the supernatant liquid into the colorimetric H-ion vessels and immediately determine its H-ion concentration by comparison with suitable colorimetric standards.

COLLABORATORS

The following ten chemists were invited to enter the collaborative work:

M. J. Blish, Univ. of Nebraska, Lincoln, Neb.
C. O. Swanson, Kansas State College, Manhattan, Kan.
A. E. Curtis, Midland Four Mills Co., Kansas City, Mo.
Wm. Green, Bakeries Service Corp., Kansas City, Mo.
C. H. McIntosh, C. J. Patterson Corp., Kansas City, Mo.
A. W. Meyer, W. E. Long Co., Chicago, Ill.
C. G. Harrel, Commander Larabee Corp., Minneapolis, Minn.
Leslie R. Olsen, International Mfg. Co., Minneapolis, Minn.
M. A. Gray, Pillsbury Flour Mills Co., Minneapolis, Minn.
Mary M. Brooke, Purity Bakeries Corp., Chicago, Ill.

Five of these chemists were equipped for collaborative work. On September 10 samples of clear and patent flours were sent to the five collaborators, who were instructed regarding the collaborative work. Each chemist was requested to run his clear and patent flour samples by both electrometric and colorimetric methods. The results are given in Table 1.

TABLE I

Collaborative results

COLLABO- RATOR NO.	ELECTROMETRIC PATENT	CLEAR	COLOR STANDARD USED	WHEN STANDARD- IZED LAST	COLORIMETRIC PATENT	CLEAR
	pH	*pH*			*pH*	*pH*
1			Liquid Para Nitrol Phenol	2 yrs. ago	5.9	6.0
2	5.67	5.91	Liquid Brom Cresol Purple	Sept. 21, '32	5.7	5.9
3	5.87	6.00	Glass Chlor Phenol Red		5.85	5.95
4	5.95	5.99	Clark & Lubs phthalate buffer solutions. Indicator used not given	Sept. 21, '32	5.9	6.0
5	5.88	6.04	Liquid Brom Cresol Purple	Sept. 21, '32	5.9	6.2
Average	5.84	5.98			5.85	6.01
Max.	5.95	6.04			5.9	6.2
Min.	5.67	5.91			5.7	5.9
Diff.	0.28	0.13			0.2	0.3

The collaborators commented as follows:

(1) We have not used the colorimetric method to any extent, but feel that it has decided advantages over the potentiometer, and it is therefore very desirable to establish a uniform or standard method of procedure.

(2) The colorimetric method for determining pH is quite satisfactory for ordinary routine work if checked frequently, electrometrically. It is desirable in colorimetric determinations to work with clear solutions. With this in mind, we made limited experiments in clarification of flour suspensions through centrifuging, and then filtering the turbid centrifuged solutions through Jena glass filtering crucibles containing fritted glass filters. The results were promising and we feel the method is worthy of further consideration.

(3) We consider the colorimetric method entirely satisfactory for mill control work.

(5) We do not use the colorimetric determination as the quinhydrone electrometric method is more dependable and just as rapid.

DISCUSSION

The average of the electrometric determinations on the patent flour was *p*H 5.84 and the average for the colorimetric was 5.85, which is remarkably close agreement.

The average of the electrometric determinations on the clear flour was *p*H 5.98 and for the colorimetric the average was *p*H 6.01, which is likewise close in agreement.

The difference between the maximum and the minimum result on the patent flour is not so large for the colorimetric method as for the electrometric method but it is very much greater in the instance of the clear flour.

In view of the different color standards used by the various collaborators and the results obtained, the agreements appear to be remarkably close.

CONCLUSION

The results secured by the collaborators indicate that the method outlined for this brief collaborative work is reasonably trustworthy and could be adopted as a tentative method of procedure. It seems advisable, however, if further study is given this method, that all collaborators use the same colorimetric standards and that they be standardized at the time of the collaborative work.[1]

REPORT ON DIASTATIC VALUE OF FLOUR

By M. J. BLISH (Agricultural Experiment Station, Lincoln, Neb.), *Associate Referee*

Last year's report,[2] based upon experiments with the Rumsey[3] type of procedure, and modifications thereof, proposed a method which the associate referee described as "having practically reached a stage where the customary type of collaborative testing should be next in order."

The work of the immediate past year may appropriately be divided into two distinct phases. The first phase was a continuation and extension of the type of studies that were pursued in the writer's laboratory in 1931 and which formed the basis of last year's report. The principal outcome of these later studies is the apparent establishment of the fact that the quantity of reducing sugars originally present as such in ordinary baker's flour is actually so small and so nearly constant as to be negligible. Substantial evidence now appears to warrant the belief that the so-called "blank" determination,—heretofore considered to be an essential feature

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 62 (1933).
[2] *This Journal*, 15, 572 (1932). See also Blish, Sandstedt and Astleford, *Cereal Chem*, 9, 378 (1932).
[3] Am. Inst. Baking, Bull. 8, 1932.

of the Rumsey type of method—is for ordinary purposes an unnecessary step which may be dispensed with. These observations and conclusions are in substantial conformity with the findings of Kent-Jones.[1]

COLLABORATIVE TESTS

The second phase of the work was the collaborative study. These tests involved eight collaborators and seven laboratories, two of the collaborators working independently in the same laboratory. The statement of the method, and the notes and comments furnished each collaborator, will not be published at this time because they vary but slightly from those given in last year's report.

Each collaborator received two samples of flour, marked, respectively, A and B. A was unquestionably a high diastatic flour, while B was lower than the average in diastatic power. Blank determinations were omitted. The collaborative results are given in Table 1.

DISCUSSION

Although the collaborative results are obviously far from satisfactory, there are several instances of reasonably good agreement when it is considered that a biochemical procedure involving enzyme action is being dealt with, and that several of the collaborators were handling the specified method for the first time.

Reasons for discordant results in certain cases are difficult to identify. For instance, A's results were nearer the average with the copper reduction method than with the colorimetric procedure. The results secured by H, however, were in quite the opposite direction. H's results with the Munson-Walker copper method obviously are decidedly erroneous, whereas B, D, and F, who also followed the Munson-Walker method, obtained fairly consistent and satisfactory results.

If the factor of economy in time and labor involved is disregarded, and only reliability and concordance of results among different collaborators is considered, the data afford no adequate basis for preference as between the colorimetric and copper reduction methods. The majority obtained slightly higher values with the colorimetric than with the copper method, although with two collaborators the reverse was true.

CONCLUSIONS, COMMENTS, AND RECOMMENDATIONS

The collaborative results, together with some of the collaborators' special comments, and further experiences on the part of the associate referee are of considerable value in indicating certain items that should be stressed in future work on this project.

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[1] Modern Cereal Chemistry. The Northern Publishing Co. Liverpool (1922).

TABLE 1

Results of collaborative diastatic activity tests. (Numerical values represent mg. of maltose per 10 grams of flour after 1 hour's diastasis.)

COLLABORATOR		SAMPLE A		SAMPLE B	
		METHOD		METHOD	
		COLORIMETRIC	COPPER	COLORIMETRIC	COPPER
A[1]		350	315	174	158
		380	335	168	156
		384	331	174	155
	Av.	371	327	172	156
B[2]			349		151.8
			347		152.8
					152.0
	Av.		348		152
C[2]		303	359	147	183
		294	361	145	180
	Av.	299	360	146	182
D[2]		353	312	157	148
E[3]		345	316	147	137
F[2]		339	319	147	149
G[2]		276	289	123	155
		316	307	119	153
	Av.	296	298	121	154
H[2]		400	222	160	252
		400	227	160	257
		369		155	
	Av.	390	225	158	255
General Average		342	313	150	167

[1] Used volumetric copper method of Lane & Eynon, *J. Soc. Chem Ind* , **42**, 32–37 (1920).
[2] Munson-Walker copper method used.
[3] Used copper method of Quisumbing and Thomas, *J. Am. Chem. Soc.*, **43**, 1503–1525 (1921).

First of all, the *p*H of the citrate buffer solution should have been noted in the specifications sent to collaborators, as this would have enabled them to check their solutions in case of doubt. Experience has shown that it is relatively easy to make errors of calculation in preparing this buffer solution and that such errors may cause decidedly erroneous diastatic values. The *p*H should be 4.6–4.8.

It may be found desirable to specify that the buffer solution be satur-

ated with toluol, in order to prevent the subsequent growth of molds in the buffer solution and to preclude any possibility of bacterial action during diastasis in the flour suspension.

For the picrate colorimetric estimation of maltose it is probable that a dextrose standard should be substituted for the maltose standard, in view of decidedly greater ease and economy with which pure dextrose can be obtained. (See Bureau of Standards Sample No. 41.) This, however, would doubtless necessitate the introduction of a factor to correct for a difference in the reducing values of dextrose and maltose.

There is the further possibility that the colorimetric procedure can never be satisfactorily adapted to collaborative requirements, and that certain volumetric copper reduction methods, such as the procedures of Lane and Eynon and of Benedict,[1] should be given consideration. Any copper reduction procedure, however, requires relatively a much larger quantity of extract than does the colorimetric method, and this situation introduces a filtration problem. Some flour extracts filter much more slowly than others. Thus, nearly all collaborators noted that Sample B filtered much more rapidly (and gave a clearer filtrate) than Sample A. This phase needs further consideration and study, because any considerable delay at that stage of the procedure is likely to involve an appreciable hydrolysis of sucrose by the acid that is added to inactivate the diastase. It would be desirable if a volumetric copper method could be applied without filtration, through paper, or without centrifugation, but with merely pouring off some of the supernatant liquid through a loose cotton plug.

It is the opinion of the associate referee that reasonable progress has been made. It is recommended[2] that further collaborative studies be undertaken with special reference to the comments and suggestions that have been based upon the findings presented in this report.

SUPPLEMENTARY REPORT

Subsequent to the preparation of the foregoing report, the associate referee established a procedure for the estimation of maltose in small quantities of flour extracts which is an adaptation of the Hagedorn-Jensen micro method for estimating blood sugar.[3] This method has distinct and conspicuous advantages over the colorimetric method, as well as over any of the various copper reduction methods that have been considered. Among the advantages offered by the modified Hagedorn-Jensen procedure are the following:

1. The method is simpler, shorter, and more convenient than any colorimetric or copper reduction procedure. No colorimeter or other special equipment is needed. No standard sugar solutions are required.

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[1] *J. Am. Med. Assoc.*, **57**, 1193 (1911).
[2] For report of Subcommittee C and action of the Association, see *This Journal*, **16**, 63 (1933).
[3] *Biochem. Z.*, **153**, 46–58 (1923).

2. Only 5 cc. of flour extract is needed, and a loose cotton plug meets all essential filtering requirements. This saves time in filtration and eliminates dangers arising from instances of slow filtration when larger quantities of extract are required, as in the Munson-Walker Method.

3. The iodine titration provides a sharp, unmistakable end point, which is sensitive to one drop of 0.05 N $Na_2S_2O_3$.

4. The entire operation, including the 1 hour digestion period, can be completed in about 1½ hours, and but little attention on the part of the technician is required.

5. All specifications must be strictly adhered to because the values are affected by the following factors: (a) Concentration of $K_3Fe(CN)_6$ solution, (b) alkalinity of solution, (c) time and temperature of heating, (d) volume of reaction mixture, and (e) kind and quantities of clarifying and enzyme-inhibiting agents used.

Specifications for the newly-proposed procedure are herewith presented. The associate referee is convinced that collaborative testing will prove this method to be decidedly superior in all respects to any that have hitherto been considered for the purpose at hand. In the writer's laboratory it has been found to yield accurate, reliable, and easily reproducible results, and it should be far more "fool-proof" than any method previously considered. It is recommended that the method be subjected to collaborative trial.

The method follows:

ESTIMATION OF DIASTATIC ACTIVITY OF FLOUR

(Revised)

REAGENTS

(a) *Buffer solution.*—Make up 3 cc. of glacial acetic acid and 4.1 grams of *anhydrous* sodium acetate to 1 liter with water. The pH of this solution is 4.6–4.8.

(b) *Sulfuric acid.*—Dilute 10 cc. of H_2SO_4 to 100 cc. with water (10 per cent by volume).

(c) *Sodium tungstate solution.*—12 per cent of $Na_2WO_4 \cdot 2H_2O$.

(d) *Alkaline ferricyanide solution.*—16.5 grams of pure dry $K_3Fe(CN)_6$ and 22 grams of *anhydrous* Na_2CO_3 in 1 liter of water. The $K_3Fe(CN)_6$ normality is 0.05 N. This solution maintains its strength for a long period of time if kept in a dark glass bottle away from the light. (The best C.P. grade of this salt purchased on the market may ordinarily be depended upon to be free from moisture and impurities to the extent that it is necessary merely to weigh out the exact quantity required for any specified normality.)

(e) *Sodium thiosulfate solution.* 0.05 N.—12.41 grams of $Na_2S_2O_3 \cdot 5H_2O$ per liter. Select only the clear crystals from the best C.P. grade. If re-distilled CO_2-free water (the second distillation being made after the addition of a small quantity of alkaline potassium permanganate to the water, to destroy all traces of organic matter) is used in making up this solution, it will retain its normality for a long time, whereas with ordinary distilled water it is likely to deteriorate slowly on standing. (Here, as in the case of the ferricyanide, it is ordinarily necessary merely to weigh accurately the quantity specified, owing to the high degree of purity in which the crystalline $Na_2S_2O_3 \cdot 5H_2O$ may be obtained. Check the ferricyanide against the thiosulfate solution as follows: To 10 cc. of the alkaline ferricyanide solution add 25 cc. of the acetic acid reagent (f) followed by 1 cc. of 50 per cent KI and 2 cc. of soluble starch solution. Titrate with the $Na_2S_2O_3 \cdot 5H_2O$ solution. It should require exactly 10 cc.

of the Na₂S₂O₃ to completely discharge the blue starch-iodine color. The Na₂S₂O₃·5H₂O solution may be standardized against pure iodine solution if necessary.)

(f) *Acetic acid solution.*—200 cc. of glacial acetic acid, 70 grams of KCl, and 20 grams of ZnSO₄·7H₂O per liter.

(g) *Potassium iodide solution.*—50 per cent solution of KI. Add one drop of concentrated NaOH for each 100 cc. of solution to prevent or substantially delay deterioration of the solution (with liberation of iodine) on standing, which will otherwise occur. The solution is not fit for use unless colorless.

(h) *Soluble starch solution.*—1 per cent of soluble starch in 30 per cent NaCl solution. Prepare soluble starch suspension and pour slowly into boiling water. Add salt and make to volume. The solution should be transparent and colorless.

<div align="center">PROCEDURE</div>

(Total Maltose after Diastasis for 1 Hour)

Introduce 5 grams of flour and a teaspoonful of ignited quartz sand into a 100 or 125 cc. Erlenmeyer flask, and mix flour with sand by rotating the flask. Add 46 cc. of buffer solution, and again mix by rotating the flask until all the flour is thoroughly in suspension. (The flask and all ingredients should be *individually* brought to 30° before being mixed together.) Digest for 1 hour at 30°, preferably in an accurately controlled water thermostat, shaking the flask (by rotation) every 15 minutes. At the end of the hour add 2 cc. of the 10 per cent (by volume) sulfuric acid solution, and mix thoroughly. Then add 2 cc. of the 12 per cent sodium tungstate solution, mix, and let stand a minute or two. Filter through paper (No. 4 Whatman or its equivalent), discarding the first 8 or 10 drops, and pipet 5 cc. of the filtered extract into a test tube of approximately 50 cc. capacity (18–20 mm. diameter). Add exactly 10 cc. (with pipet) of the alkaline 0.05 *N* ferricyanide solution to the 5 cc. of extract in the test tube, and immerse the test tube in a vigorously boiling water bath (the surface of the liquid in the test tube should be 3 or 4 cm. below the surface of the boiling water.'(The delay between the filtering of the extract and the treatment in the boiling water bath should not be more than 15–20 minutes, at the most. Further delay may cause a slight error due to sucrose hydrolysis in the acid solution.) Allow the test tube to remain in the boiling water bath for *exactly* 20 minutes. Then cool the test tube and its contents under running water, and pour at once into a 100 or 125 cc. Erlenmeyer flask. Rinse out the test tube with 25 cc. of the acetic acid solution (reagent f), and add to the contents of the Erlenmeyer flask, with thorough mixing. Then add 1 cc. of the 50 per cent KI solution followed by 2 cc. of the soluble starch solution, and mix thoroughly. Titrate with 0.05 *N* sodium thiosulfate to the complete disappearance of the blue color. A 10 cc. buret is recommended for dispensing the 0.05 *N* sodium thiosulfate in this titration. Subtract the number of cc. of 0.05 *N* sodium thiosulfate used in the titration from 10, which gives cc. of 0.05 *N* ferricyanide reduced to ferrocyanide by the reducing sugars in the flour extract. This value represents a definite quantity of maltose, which may be ascertained by consulting the Maltose Conversion Table. The table was prepared by applying the specified procedure to standard solutions of pure maltose and using all reagents in the quantities and volumes precisely as employed for flour extracts. The maltose values are given in milligrams. When 5 cc. of flour extract is used, as herein specified, it is necessary merely to multiply the mg. of maltose by 20 to give mg. of maltose per 10 gram of flour in 1 hour's diastasis. This is the value that is recorded and reported as the measure of the diastatic value of the flour in question.

The foregoing specifications may be used with all ordinary flours whose values for mg. of maltose produced by 10 grams of flour in 1 hour will seldom, if ever, ex-

ceed 350. For material giving higher values, such as products from malted or sprouted grain, use smaller portions of extract, i.e., 1, 2, or 3 cc. instead of 5 cc. In such cases, however, add enough distilled water to make up the difference, and use a different factor for converting results into mg. of maltose per 10 gram of flour. Thus, when 2 cc of extract is used, multiply the value obtained from the table by 50 instead of 20. If the material in the test tubes is colorless instead of yellow, after treatment in the boiling water bath, and gives no blue color upon the addition of KI, it is apparent that there was more than enough maltose to reduce all the ferricyanide, and that the determination must be repeated with a smaller quantity of extract.

THE "BLANK" DETERMINATION

A "blank" determination, designed to indicate the quantity of reducing sugar originally present in the flour,—the value for which presumably should be deducted from the total maltose value after 1 hour's diastasis,—has been generally regarded as an essential step in the estimation of flour diastatic activity. This operation, however, is ordinarily unnecessary when dealing with flour milled from *sound* wheat, because the quantity of reducing sugars originally present as such is so small and so nearly constant that it may be disregarded for all practical purposes. The "blank" determination may therefore be conveniently omitted in ordinary routine testing. It need be used only when there is occasion to doubt the soundness of the wheat, or in cases where there is known to have been an appreciable quantity of frosted,

Maltose conversion table

0 05 N FERRI-CYANIDE REDUCED	MALTOSE EQUIVALENT	0 05 N FERRI-CYANIDE REDUCED	MALTOSE EQUIVALENT	0 05 N FERRI-CYANIDE REDUCED	MALTOSE EQUIVALENT	0 05 N FERRI-CYANIDE REDUCED	MALTOSE EQUIVALENT
cc.	mg.	cc	mg.	cc	mg	cc	mg.
0.1	0.2	2.6	4.2	5.1	8.3	7.6	12.3
0.2	0.3	2.7	4.4	5.2	8.4	7.7	12.5
0.3	0.5	2.8	4.5	5.3	8.6	7.8	12.7
0.4	0.6	2.9	4.7	5.4	8.7	7.9	12.9
0.5	0.8	3.0	4.9	5.5	8.9	8.0	13.0
0.6	1.0	3.1	5.0	5.6	9.1	8.1	13.2
0.7	1.1	3.2	5.2	5.7	9.2	8.2	13.4
0.8	1.3	3.3	5.3	5.8	9.4	8.3	13.5
0.9	1.5	3.4	5.5	5.9	9.6	8.4	13.7
1.0	1.6	3.5	5.7	6.0	9.7	8.5	13.9
1.1	1.8	3.6	5.8	6.1	9.9	8.6	14.0
1.2	1.9	3.7	6.0	6.2	10.0	8.7	14.2
1.3	2.1	3.8	6.2	6.3	10.2	8.8	14.4
1.4	2.3	3.9	6.3	6.4	10.4	8.9	15.6
1.5	2.4	4.0	6.5	6.5	10.5	9.0	14.8
1.6	2.6	4.1	6.6	6.6	10.7	9.1	15.0
1.7	2.8	4.2	6.8	6.7	10.9	9.2	15.2
1.8	2.9	4.3	7.0	6.8	11.0	9.3	15.4
1.9	3.1	4.4	7.1	6.9	11.2	9.4	15.6
2.0	3.2	4.5	7.3	7.0	11.3	9.5	15.9
2.1	3.4	4.6	7.5	7.1	11.5	9.6	16.1
2.2	3.6	4.7	7.6	7.2	11.7	9.7	16.5
2.3	3.7	4.8	7.8	7.3	11.8	9.8	17.0
2.4	3.9	4.9	7.9	7.4	12.0	9.9	—
2.5	4.1	5.0	8.1	7.5	12.2	10.0	—

sprouted, heat-damaged, or otherwise unsound kernels in the wheat from which the flour was milled.

When, however, it is considered necessary to make the blank determination, proceed as follows: Add to 5 grams of flour and a teaspoonful of quartz sand in a 100 or 125 cc. Erlenmeyer flask 48 cc. of 0.4 per cent (by volume) H_2SO_4. (It is recommended that the 0.4 per cent H_2SO_4 be pre-cooled to ice-water temperature before it is added to the flour. The error is very slight, however, if this step is omitted.) Shake the mixture thoroughly, and at once add 2 cc. of 12 per cent sodium tungstate; shake thoroughly again and, after allowing to stand 2 minutes, filter through a No. 4 Whatman (or its equivalent) paper. Using 5 cc. of the clear filtrate, proceed according to the specifications for the diastatic activity previously described.

REPORT ON STARCH IN FLOUR

By LLEWELYN JONES (U. S. Food and Drug Administration, Kansas City, Mo.), *Associate Referee*

Last year's report on the determination of starch in flour[1] showed the necessity of further study of the two methods involved. It was believed that if the tentative method (Rask)[2] was modified so as to give a more uniform dispersion of the starch in the hydrochloric acid and if means were devised to hasten the filtrations, more concordant results would follow.

V. E. Munsey made a rather extensive study of the tentative method and so modified it as to overcome, apparently, the objections mentioned above. He submitted the modified method for collaborative study and the results of the collaborators are included in this report. The associate referee also made further study of the tentative method as modified by Munsey and obtained satisfactory results.

Two commercial brands of flour, a white patent and a whole wheat flour, were submitted to ten collaborators. A copy of the modified tentative method was submitted to each collaborator, with instructions to determine the starch in the samples according to the methods submitted and also by the diastase-acid hydrolysis (Hartmann-Hillig) method.[3]

MODIFIED RASK METHOD FOR STARCH

REAGENT

Dilute hydrochloric acid.—Mix approximately equal volumes of strong HCl and H_2O and adjust by titration so that 100 cc. of the solution contains 20.5–21.0 grams of HCl.

DETERMINATION

Weigh accurately a sufficient quantity of finely ground sample (should readily pass through a 20-mesh sieve) to represent 0.5–1.0 gram of starch. The quantity of starch finally weighed will then vary from 0.25–0.5 gram. Transfer to a funnel fitted with a 9 cm. S and S No. 589 White Ribbon or Whatman No. 40 filter paper and extract by nearly filling the filter four times with ethyl ether, likewise extract with 70 per cent alcohol (by volume) and with H_2O. Allow to drain one hour uncovered.

[1] *This Journal*, 15, 582 (1932).
[2] *Methods of Analysis, A.O.A.C.*, 1930, 172.
[3] *This Journal*, 14, 112 (1931).

Transfer the drained filter and contents to a 50 cc. beaker. The stirring rod to be used in the next step should have a flattened end 15 mm. in diameter. (It is very important to tamp with a twisting motion during the time specified in order to get the filter paper completely disintegrated and thus insure the complete suspension of the starch in the hydrochloric solution. This time should be sufficiently long and the

TABLE 1

Results of collaborators on samples submitted by V. E. Munsey

COLLABORATORS	SAMPLE NO. 1—PATENT FLOUR			SAMPLE NO 2 —WHOLE WHEAT FLOUR*		
	per cent	*per cent*	Av	*per cent*	*per cent*	Av.
J. H. Loughrey New York, N. Y.	74.22	74.30	74.26	57.32 57.32	57.08	57.31
— — Smelzer Lockport, N. Y.			73.60			58.85
E. H. Berry Chicago, Ill.	74.30	73.84	74.07	57.68	57.74	57.71
J. H. Bornman Chicago, Ill.	73.26 73.60	74.24 73.84	73.73	57.32 56.68 57.58 56.92		57.12
A. K. Klein San Francisco, Calif.	72.92	73.84	73.38	57.26 57.90		57.58
V. E. Munsey Washington, D. C.	73.80 73.84 73.84	74.16 74.24	73.98		58.24 58.28 58.44 58.28 58.52	58.32
Sample from another jar of same whole wheat flour					57.58 57.84	57.86
Average of all analyses			73.84			57.82
L. Jones† Kansas City, Mo.	73.48 73.64 73.48			57.28 57.52		

* It is very difficult to get a sample of whole wheat flour sufficiently well mixed so that the floury material is uniformly distributed throughout a given sample. This sample was very finely ground and was as uniformly mixed as is possible for a sample of this nature.
† Received too late to be included in the average.

maceration complete to allow the suspension of all the starch but not to hydrolyze any of it. Maceration should be completed while there is a small amount of HCl present and the whole contents is a rather thick paste. If this optimum condition is obtained practically duplicate results will follow. Add the hydrochloric acid reagent at 15°C. to the beaker containing the sample, using a fast delivering 10 cc.

Mohr pipet with 1 cc. marked off at the lower end with heavy pencil marks. Keep the acid supply on the bench, but do not allow it to get above 18°C.

Proceed as follows, adding the HCl in the quantities given: Add 1 cc., tamp 1 min.; add 1 cc., tamp 2 min.; add 1 cc., tamp 2 min.; add 1 cc., tamp 1 min.; add 1 cc., tamp 1 min.; add 1 cc., tamp 1 min.; add 1 cc., tamp 1 min.

Fill beaker half full and stir 30 seconds.

Fill beaker three-fourths full and stir 30 seconds.

For 10 minutes during this treatment the paper should be completely disintegrated and in a smooth state of suspension, the tamping should be continued vigorously during this time, and as little time as possible should be spent adding the acid. Transfer immediately to a 100 cc. wide mouthed volumetric flask, rinsing out the beaker; carefully make to volume with HCl reagent; and add 0.5 cc. for volume of filter paper (this step requires 2 minutes). Next shake the stoppered flask vigorously for 5 minutes, then allow to stand 5 minutes in a beaker of H_2O at 20°C. Shake a couple of times and filter immediately into a 250 cc. suction flask through a small Büchner funnel (41 mm. in diameter) fitted with a thin layer of asbestos and filled half full with dry, fluffy asbestos. The filtration requires 1 minute only. Pipet immediately 50 cc. of the filtrate into a 200 cc. beaker (tall form) containing 115 cc. of 95 per cent alcohol (by volume). (The time consumed from the initial addition of the acid to the sample is 24 minutes.) Allow the pipet to drain completely and then stir with a whipping motion for 1 minute to flocculate the precipitated starch. Wash down sides of the beaker with 70 per cent alcohol. Allow to stand 3–4 minutes, until nearly all the precipitate has settled, and then carefully decant the supernatant liquid, which is somewhat turbid, so that little or no precipitate passes into the weighed Gooch crucible, which has been fitted with a thin pad of ignited asbestos and is half filled with fluffy ignited asbestos. Wash the precipitate by decantation, using successively two 40 cc. portions of 70 per cent alcohol (by volume) then four times, using about 30 cc. portions of 95 per cent alcohol (by volume), each time breaking up the precipitate by rapid stirring and allowing the precipitate to settle before decantation. After each stirring rinse the sides of the beaker with a small stream of alcohol to prevent the starch from drying and sticking to the sides of the beaker. Finally transfer the starch completely by means of a jet of 95 per cent alcohol (by volume) and wash the sides of the Gooch and precipitate with a little of the alcohol. (All these filtrations are very fast.) Dry the crucible and contents uncovered for 2 hours at 130°C., place in a desiccator charged with P_2O_5, or fresh concentrated H_2SO_4, cover immediately, cool 10 minutes, and weigh. Multiply result by two and report as starch. These directions must be followed carefully in every detail to obtain satisfactory results. Since the steps are timed it is essential to learn the procedure and not attempt to ponder over it to find out the next steps. Arrange everything needed in the determination before the HCl is added to the sample.

COMMENTS BY COLLABORATORS

C. B. Morison.—No difficulties were experienced at any stage of the method. . . . As far as duplication of results goes, this procedure appears to be more satisfactory than those previously studied.

J. H. Loughrey.—The Rask method, after a little experience, is rapid and it seems to me, accurate.

V. E. Munsey.—These results seem to be about what would be expected of the two methods. On the whole wheat, the starch is much greater by the Hartmann and Hillig method. It seems the more bran there is in the product the greater the difference in starch by the two methods, which is to be expected since the percentage

of pentosans has been increased. In view of the fact that the Hartmann and Hillig method gives pentosans along with the starch, it seems to me enough time has been devoted to this method.

Gordon Smith.—The results obtained with the two methods seem to agree more closely in the case of white flour than in that of whole wheat. The modified Rask method seems to give better checks between duplicates of the same sample.

TABLE 2

Results of collaborators on samples submitted by the associate referee

COLLABORATOR	PATENT FLOUR				WHOLE WHEAT FLOUR			
	MODIFIED TENTATIVE METHOD		DIASTASE-ACID HYDROLYSIS METHOD		MODIFIED TENTATIVE METHOD		DIASTASE-ACID HYDROLYSIS METHOD	
	per cent	Av.	per cent	Av.	per cent	Av.	per cent	Av
C. B. Morison	71.36 71.48	71.42						
J. H. Loughrey	73.34 73.14	73.24			55.32 54.72	55.02		
V. E. Munsey	72.52 72.42 72.78	72.57	73.47 73.58	73.53	55.24 55.08 55.52 55.60	55.36	59.68 59.96	59.82
Gordon Smith	72.14	72.14	74.59	74.59	55.02	55.02	60.61	60.61
C. B. Stone	74.04 72.90 73.10	73.35	71.56 72.93 72.66 73.05	72.55	55.87 56.09 55.72	55.89	59.27 60.31 60.26	59.95
L. Jones	72.76 72.50 72.34	72.53	72.73 72.39	72.56	55.16 54.84 55.36	55.12	60.26 59.85	60.05
Average of all analyses		72.54		73.31		55.28		60.11
Maximum		74.04		74.59		56.08		60.61
Minimum		71.36		71.56		55.02		59.27

C. B. Stone.—The only comment on the Hartmann and Hillig method is in regard to the 70 per cent alcohol. The method calls for 70 cc. of 95 per cent alcohol made to 100 cc. and refers to 70 per cent alcohol throughout the method. This does not seem to be an exact statement.

The Rask method does not offer any particular difficulty and is much shorter than the Hartmann and Hillig method. The only questions are the following: Is all the residue thrown out by the alcohol pure starch? How much starch is actually lost in this procedure? The results secured by both methods are far from being good checks.

DISCUSSION

Although the results are somewhat disappointing, comments by the collaborators indicate that no special difficulties were experienced with the methods.

The results obtained by the modified tentative method show some improvement over those obtained last year by the tentative method. It is believed that the variation in the results reported is due to the unfamiliarity of the analyst with the method. If the directions given in the method are followed closely it is believed that with a little experience the analyst will be able to secure reasonably concordant results.

The diastase-acid hydrolysis method gives higher results than the modified tentative method; this is especially true in the case of whole wheat flour. This method is somewhat complicated and time-consuming and offers considerable chances for errors. The copper reduction is made on a small aliquot, and any error which may have been introduced is greatly multiplied in the final calculations.

RECOMMENDATIONS[1]

It is recommended—

(1) That the modified tentative method be given further collaborative study.

(2) That study of the diastase-acid hydrolysis method be discontinued.

REPORT ON FLOUR-BLEACHING CHEMICALS

By DOROTHY B. SCOTT (U. S. Food and Drug Administration,
New York, N. Y.), *Associate Referee*

Subcommittee C recommended that the Seidenberg method for determining chlorine in chlorine-bleached flour as modified by Spencer[2] be further studied and special attention given to making the end point sharper.

J. L. Hogan of the New York Station ran a series of 34 flours, using the Seidenberg method. From actual experience it was found that the modification proposed by Spencer, using ammonium or sodium bicarbonate to prevent the alkali from attacking the platinum, was not necessary if the precaution of using a small yellow flame of a Bunsen burner given in the method was carefully followed. It was impossible to obtain ammonium or sodium bicarbonate without a high chloride content. As the blanks were extremely high, anyway, the method was used without the modification. The eight platinum dishes used for the duplicate determinations and blanks of the 34 samples did not lose any more in weight than in ordinary usage. Two dishes lost 0.01 gram and the others less during six months' constant use.

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 62 (1933).
[2] *This Journal*, 14, 486 (1931).

Several difficulties were encountered. The method is time-consuming, requiring about twice as much time as the method used later. It was difficult to obtain a small blank, the range being from 16 parts per million to 109 parts per million of chlorine in the reagents. Experiments with the various reagents used showed that the high blanks were probably due to the sodium. When a sodium with the lowest percentage of chlorine obtainable was used, the blank was 67 parts per million. It was difficult to detect the end point even after considerable experience with the method.

H. D. Grigsby suggested that the Rask method for the determination of chlorine be changed by using the Volhard method of titration with stronger solutions than those employed in the Seidenberg method. The Volhard method has been preferred to the Mohr method by two former associate referees, G. C. Spencer[1] and A. Seidenberg.[2]

The Rask method given in *Methods of Analysis, A.O.A.C.*, 1930, as "Tentative Method II" for the determination of chlorine in wheat flour was followed to the titration, except that a 110 cc. volumetric flask was substituted for a 300 cc. volumetric flask for the collection of the solution after ashing.

The following reagents were used:

Ferric alum indicator.—To a cold saturated solution of ferric-ammonium alum add sufficient nitric acid to cause the disappearance of the brown color.

Standard silver nitrate solution.—4.791 grams of silver nitrate per liter. 1 cc. = 1 mg. of chlorine.

Standard potassium thiocyanate solution.— 2.7405 grams of KCNS per liter. 1 cc. = 1 cc. of standard silver nitrate solution.

Change the Rask method at "Neutralize the acidity" to "Add 10 cc. of AgNO₃ solution to the flask. Shake and add water to the 100 cc. mark. Place the flask in boiling water for about 5 minutes. When the contents are cool, make up to the second mark with water, shake and filter. Place 100 cc. of the clear filtrate in a porcelain casserole. Add 2 cc. of ferric alum indicator. Titrate to a faint orange color with the potassium thiocyanate solution. Run blanks on all the reagents used and report chlorine on a dry basis."

This method eliminated the three difficulties encountered in using the Seidenberg method: length of time necessary for its use, the high blanks, and the difficult end point.

A few samples were compared and the following results were obtained:

Chlorine (parts per million)—analyses by Hogan

	SEIDENBERG METHOD		MODIFIED RASK METHOD	
	BLANK	SAMPLE	BLANK	SAMPLE
1.	77.3	142.4	13.6	138.9
		121.1		
		Av. 131.8		
2.	77.3	112.4	16 0	159 7
		111.8		149.2
		Av. 112.1		Av. 154.5

[1] *This Journal*, 11, 487 (1928).
[2] *Ibid.*, 132.

3.	77.3	104.6	16.0	143.0
		102.1		132.6
		Av. 103.4		Av. 137.8
4.	77.3	16.6	16.0	2.8
		11.2		2.8
		Av. 13.9		Av. 2.8

A commercial sample of flour was analyzed by this method by J. L. Hogan and another sample of the same flour was analyzed two weeks later by the associate referee. The results are as follows:

Chlorine (parts per million)

	BLANKS	SAMPLE
J. L. Hogan	13.2	63.8
	13.2—Av. 13.2	63.8—Av. 63.8
D. B. Scott	12.0	66.0
	12.0—Av. 12.0	66.0—Av. 66.0

Not enough determinations were made to justify a definite statement as to which is the more accurate method. However, the results do not vary as much as did those of the collaborators using the Seidenberg method. It is recommended that more study be given to a comparison of the two methods.

A modification of the Rothenfusser method[1] for the detection of benzoyl peroxide in flour was reported last year. It has been found impossible, however, to obtain the reagent para-diaminodiphenylamine sulfate in the United States. The base para-diaminodiphenylamine may be used successfully by adding two drops of concentrated H_2SO_4 to the 0.05 per cent alcohol solution of the base. The associate referee is working on a method for the detection of benzoyl peroxide as benzoic acid.

It is recommended[2]—

(1) That the proposed modification of the Rask method for the determination of chlorine in flour be studied further.

(2) That the method for the detection of benzoyl peroxide as benzoic acid be submitted for collaborative study.

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No report on foreign methods for testing flours was given by the associate referee.

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[1] *Chem. Ztg.*, **39**, 285 (1925).
[2] For report of Subcommittee C and action of the Association, see *This Journal*, **16**, 63 (1933).

REPORT ON CARBON DIOXIDE IN SELF-RISING FLOURS

By L. D. WHITING (Ballard and Ballard Co., Louisville, Ky.),
Associate Referee

Last year's collaborative work[1] on the determination of carbon dioxide in three samples of self-rising flour showed that the method submitted gave results lower than the theoretical carbon dioxide content. As these results did not seem entirely satisfactory, it was recommended that further experimental work, together with a limited amount of collaboration, be conducted on the methods for the determination of carbon dioxide in self-rising flour.

Some of the experimental work was done, but owing to press of other work during the past year it was not possible to carry out the suggested collaborative work. This study shows that there are several factors which exert an influence on the results obtained by the gasometric method.

Attention is called by Hertwig and Hicks[2] to one of these factors, namely, that the addition of a measured volume of reagent-acid to the dry decomposition flask causes a greater displacement in the gas measuring tube than does the volume of added acid. This displacement, they state, is greater by about 3 to more than 5 cc., depending on the barometric pressure, the temperature, and the acid concentration, and is attributable to the vapor pressure of the reagent-acid.

Another factor opposed to this one is the quantity of carbon dioxide which remains dissolved in the liquid in the reaction flask.

Chittick, Dunlap, and Richards[3] point out that in the analysis of a baking powder by the official gasometric method for carbon dioxide in baking powder the results obtained are very closely in agreement with the actual total carbon dioxide present. They find that whatever vapor tension effect is produced by the liquid in the reaction flask is compensated for by the carbon dioxide dissolved in the liquid.

The associate referee likewise pointed out last year that the flour-acid mixture in the flask holds a small percentage of carbon dioxide and that the results obtained on self-rising flours by the method suggested are slightly low. These results range from 93.8 to 95.5 per cent of the theoretical carbon dioxide content, and are the net result of the effect of the several opposing factors, which are only partially compensatory in the case of self-rising flour. The conclusion must be drawn that in this case the increased displacement in the volume buret due to the vapor pressure is more than offset by the amount of carbon dioxide dissolved in the liquid, and the net result is slightly low.

The partial results of the experimental work carried on this year show

[1] *This Journal*, 15, 588 (1932).
[2] *Cereal Chem.*, 5, 482 (1928).
[3] *Ibid.*, 7, 473 (1930).

that there is no significant difference in the amounts of carbon dioxide remaining in the liquid when two different flours are used. They also show that the volume reading due to vapor pressure in case of the dry flask is, for example, 3 cc., but that in the case of the dry flask containing approximately 17 grams of plain flour the reading is greater—for example, 5 cc. This may mean either that the reading due to the vapor pressure of the flour-acid mixture is different than that due to the vapor pressure of the reagent-acid alone, or that the carbon dioxide blank of the plain flour causes the difference. The experimental work also shows that the method suggested gives results slightly lower than the theoretical carbon dioxide content.

The associate referee thinks that it may be feasible to determine and use a factor which would be applied to results obtained by this method and which would give figures in close agreement with the theoretical carbon dioxide content. From the work done to date it is not possible at this time to submit a factor, but it is suggested that the next referee study and check such a factor collaboratively.

The gasometric method requires considerably less time than the absorption method. Therefore, if numerous tests are run, the ease and speed with which the Chittick apparatus may be handled make the gasometric procedure a desirable one in many laboratories.

It is recommended[1] that the methods for the determination of carbon dioxide in self-rising flour be further studied.

REPORT ON SAMPLING AND DETERMINATION OF MOISTURE IN ALIMENTARY PASTE, BREAD, AND BAKED PRODUCTS

By L. H. BAILEY (U. S. Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

The work of determining moisture in bread and baked products for the year 1932 was confined to a collaborative study of the vacuum oven and the 130°C. air-oven methods. For this purpose bread, cake, crackers, vanilla wafers, and pretzels were obtained in the open market. These products were broken into small particles, spread out in the laboratory, and allowed to dry until they came to constant weight.

The collaborative results (expressed in percentage) follow:

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 63 (1933).

Moisture in baked cereal products

COLLABORATOR	RUTH CAPEN		V. E. MUNSEY		L. H. BAILEY	
METHOD	VAC. OVEN	AIR OVEN	VAC OVEN	AIR OVEN	VAC. OVEN	AIR OVEN
Bread	9.81	9.81	9.69	9.61	9.71	9.68
	9.71	9.78	9.65	9.67	9.78	9.68
Cake	8.01	7.83	7.71	7.62	7.80	7.88
	7.91	7.86	7.62	7.65	7.83	7.92
Crackers	8.11	8.12	7.14	7.11	7.36	7.20
	8.13	8.10	7.23	7.21	7.36	7.21
Vanilla wafers	6.03	6.10	6.05	5.98	5.95	6.04
	6.07	6.01	6.08	6.05	5.99	6.10
Pretzels	8.21	8.22	7.79	7.84	7.89	7.90
	8.18	8.14	7.82	7.81	7.91	7.91

It is apparent that concordant results may be obtained by using either of these methods for the determination of moisture in air-dried baked products.

RECOMMENDATIONS[1]

It is therefore recommended—

(1) That Method I under Bread, the vacuum oven method (official, first action) for the determination of moisture in air-dried bread, be made official (final action).

(2) That Method II, under Bread, the air-oven method (official, first action) for the determination of moisture in air-dried bread, be made official (final action).

(3) That both of these methods be extended to include other baked products.

ALIMENTARY PASTES

As the tentative method for Collection and Preparation of Sample[2] has been found to be satisfactory, it is recommended that it be made official (first action).

No report on unsaponifiable matter was given by the associate referee.

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[1] For report of Subcommitte C and action of the Association, see *This Journal*, 16, 64 1933).
[2] *Methods of Analysis*, A.O.A.C., 1930, 180.

REPORT ON FAT BY ACID HYDROLYSIS, TOTAL PHOSPHORUS (P_2O_5), LIPOIDS AND LIPOID PHOSPHORUS (P_2O_5), AND WATER-SOLUBLE NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL IN FLOUR, BREAD, AND ALIMENTARY PASTES

By LLOYD C. MITCHELL (U. S. Food and Drug Administration, St. Louis, Mo.), *Associate Referee*

FAT BY ACID HYDROLYSIS

The present method for the determination of fat by acid hydrolysis, official for flour[1] and official, first action, for bread and alimentary pastes[2], was first brought to the attention of this Association by Hertwig.[3] He pointed out that the determination of fat in alimentary pastes, egg noodles, and bread by the direct extraction of the sample with dry ether gives results considerably less than those for the combined fat of the ingredients entering into these products. He thought the low results for ether extract were due to the failure of the ether to penetrate sufficiently the hard glutinous particles and the unbroken plant cells to extract all the fat. As acid with heat hydrolyzes the proteins and the starches, disrupts the cell walls, and liberates the fat, he developed a method to extract the fat from these products more completely than did the extraction with dry ether, and designated it as an "acid digestion method." He found that the vigorous acid treatment more or less destroyed the lipins in that the fat extracted contained much less lipin-phosphorus acid than the actual amount present as determined by hot alcohol extraction or other methods. Hertwig's method called for an acid treatment of the sample "at 65°, with stirring at frequent intervals for 15–25 minues, or until the proteins and starch are sufficiently hydrolyzed to form a clear solution."

In submitting the method to the collaborators Bailey[4] changed the statement to read "at 65° until particles are broken up, and everything except fibrous material is in solution. During this heating (10–15 minutes) stir. . . ." Using the method thus modified four collaborators reported fat results varying from 1.83 to 2.78 per cent on bread, and from 4.65 to 9.96 per cent on soda biscuits or crackers. In his recommendations to the Association, Referee Bailey made no reference to the acid hydrolysis method.

Alfend[5] reported the results of seven collaborators working on a sample of patent flour as varying from 1.27 to 1.60 per cent fat. The method, which he received from Referee Hertwig, read "at 70–80°, and at frequent intervals for 30–40 minutes." This wording occurs in the official method for flour. Alfend[6] reported the results of five collaborators working on a

[1] *Methods of Analysis, A.O.A.C.*, 1930, 166.
[2] *Ibid.*, 178, 181.
[3] *This Journal*, 6, 508 (1923).
[4] *Ibid.*, 6, 60 (1922).
[5] *Ibid.*, 9, 429 (1926).
[6] *Ibid.*, 10, 484 (1927).

sample of unbleached patent flour as varying from 0.42 to 0.80 per cent fat by the official direct ether extraction method and from 1.12 to 1.37 per cent fat by the acid hydrolysis method; on a sample of water noodles as varying from 0.05 to 0.34 per cent fat (ether extract) and from 1.90 to 2.27 per cent fat (acid hydroylsis); and on a sample of egg noodles as varying from 3.17 to 3.66 per cent fat (ether extract) and from 4.69 to 5.09 per cent (acid hydrolysis).

The next year Alfend[1] reported the results of six collaborators working on a sample of water noodles as varying from 0.05 to 0.34 per cent fat (ether extract) and from 1.42 to 1.60 per cent fat (acid hydrolysis); and on a sample of egg noodles as varying from 2.20 to 2.95 per cent fat (ether extract) and from 3.39 to 4.01 per cent fat (acid hydrolysis).

It is apparent from the reports of Alfend that the acid hydrolysis method for fat yields considerably higher results than are obtained by the direct ether extraction method. The variation in results by both methods however, are somewhat wide.

In 1928 Alfend[2] reported the results of six collaborators working on a sample of bread as varying from 4.16 to 4.52 per cent fat (acid hydrolysis).

Bornmann[3] reported the results for fat (acid hydrolysis) obtained by three collaborators as varying from 4.78 to 5.03 per cent on egg noodles, from 1.68 to 1.83 per cent on macaroni, and from 6.87 to 7.09 per cent on bread. It is noted that the results varied appreciably less than those reported in the previous collaborative studies.

Last year Subcommittee C recommended further study of the acid hydrolysis method for the determination of fat in bread and alimentary pastes. In applying the method for the determination of fat in eggs (16, 300) it was found necessary to raise the temperature to boiling to insure the complete splitting off of the phosphoric acid-chlorine group from the lecithin, since hydrolysis at 70–80° caused only a partial and variable splitting off of this group. As the acid hydrolysis method was designed primarily to determine fat in egg noodles, it seemed desirable to raise the temperature of the hydrolysis to that found most suitable for eggs.

Accordingly, samples of (1) patent flour, (2) water noodle made from the patent flour, (3) wheat ground in the laboratory, (4) graham flour ground in a mill from the wheat represented by (3), and (5) graham flour same as (4) except with 2 per cent of the bran removed, were run as directed by the method except that the temperature of hydrolysis was raised to boiling instead of to 70–80°. The results for fat are (1) 0.68, (2) 0.75, (3) 1.55, (4) 1.60, and (5) 1.88 per cent, respectively. The hydrolysis, which was done by inserting the Majonnier tubes into a boiling water bath, caused considerable charring. The ether-petroleum ether extract was highly colored and the fatty residue after drying was not entirely

[1] *This Journal*, 11, 495 (1928).
[2] *Ibid.*, 12, 394 (1929).
[3] *Ibid.*, 14, 489 (1931).

soluble in chloroform. The third extraction was colored nearly as much as the first extraction, indicating that the hydrolysis produced substances which were being determined as fat.

A sample of potato starch was then washed with several portions of ether to remove any fatty substances which might be present. The apparent amount of fat obtained from the washed potato starch when hydrolyzed at boiling temperature was 2.20 and 2.30 per cent; at 71–74°, 0.15 and 0.10 per cent; at 57–61°, 0.08 and 0.07 per cent; and at 51–52°, 0.04 and 0.04 per cent. The amount of fat in the starch obtained by the chloroform-alcohol method (16, 303) was 0.01 and 0.01 per cent. The hydrolysis at boiling temperature produced a large amount of carbon-like particles. The ether-petroleum ether extract was highly colored, and the dried residue appeared to be entirely soluble in chloroform. At 71–74° the ethereal layer was slightly amber color; at 57–61°, slightly colored; and at 51–52°, colorless.

The fat was then determined on samples 3, 4 and 5 identified above by hydrolysis at 45–49° with the following results: (3) 1.40, (4) 1.30, and (5) 1.00 per cent, respectively. With samples 1, 2, 3, 4, and 5 identified above, and 6, a sample of bread, the determination of fat was reported, hydrolysis being made at 48–49°. The results are: (1) 0.95, (2) 0.85, (3) 1.35, (4) 1.20, (5) 1.40, and (6) 2.70 and 2.30 per cent, respectively.

The associate referee was unable to complete the study of the method in time to make his report to the Association. The results obtained, however, indicate that the temperature of 70–80° for the hydrolysis, as now directed in the official methods is too high and the range too great to insure concordant results in the hands of different analysts. The low results obtained by hydrolysis at 48–49° indicate that the hydrolyzation temperature sufficiently low to cause no charring may not be sufficiently high to yield all the fat on extraction with ether-petroleum ether.

It is recommended that the method for determining fat in flour, baked products, and alimentary pastes by acid hydrolysis be further studied.

PHOSPHORUS

Last year Subcommittee C recommended that the method for the determination of phosphorus in eggs[1] be studied relative to its applicability to baked products and alimentary pastes. Since the Association has no method for the determination of phosphorus in flour, flour was also included in the study.

The method used for eggs appears to be satisfactory for patent flours and water noodles made from patent flours. On ground wheat and graham flours, which carry a larger percentage of phosphorus compounds than patent flours, the charred material which was discarded on eggs after

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leaching with nitric acid was found to contain varying small amounts of phosphorus. Doubling the volume of the sodium carbonate solution reduces the amount of phosphorus remaining in the charred material after it is leached with nitric acid and also causes the charred material to be more easily broken up than when the usual amount of sodium carbonate is used. When the temperature is raised from 500° to 700°, repeated tests for phosphorus in the charred material after leaching with nitric acid have been negative. It appears that the method for the determination of phosphorus in eggs is satisfactory for the determination of phosphorus in flour, baked products, and alimentary pastes provided the temperature is raised to 700°.

It is recommended that the method for the determination of phosphorus in eggs, as modified in this report, be studied collaboratively on flour, baked products, and alimentary pastes.

LIPOIDS AND LIPOID PHOSPHORUS

Bornmann[1] showed that with samples of egg noodles, macaroni, and bread the determination of lipoids by the present method, which is official (first action) for baked products and alimentary pastes, yielded 0.59, 0.42, and 0.56 per cent fat as determined by the acid hydrolysis method and the use of the air-dry residue from the lipoid extraction. He found that extracting dry alimentary pastes in the cold with a mixture of equal parts of alcohol and chloroform gave low results. After preliminary investigation he formulated a method whereby he digested a 10 gram sample in 30 cc. of 70 per cent alcohol at 75°–80° for 15 minutes, added 70 cc. of 95 per cent alcohol, cooled, and then added 100 cc. of chloroform. His results for lipoids in egg noodles and bread are appreciably higher by this method than by the official method.

The preliminary treatment of the sample—digestion at 75°–80° in 30 cc. of 70 per cent alcohol—is done with a view to getting the particles of the cereal product into a condition whereby they are penetrated by the solvent. The official method calls for a similar treatment of the sample, but Bornmann points out that the official method is not entirely satisfactory owing to the incomplete extraction of the lipoids.

In the alcohol-chloroform method for the determination of lipoids in liquid eggs (16, 303) the extraction is made without previous treatment to remove the water. This procedure suggested the possibility of extracting the lipoids from the wetted sample, the method being to add sufficient water to form a dough before extracting with the mixed solvent.

Some preliminary work was done on a sample of wheat ground to pass a 30-mesh sieve. Different trials yielded the following percentage of lipoids: 2.48, 2.58, 2.50, 2.48, 2.38, and 2.68. Aumer (private communication) reported 1.72 per cent fat obtained by the official ether extraction method.

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[1] *This Journal*, 14, 489 (1931).

While these results appear promising, lack of time prevented comparison of the method with the official method or with the method used by Bornmann.

It is recommended that the methods for the determination of lipoids in flour, baked products, and alimentary pastes be further studied.

WATER-SOLUBLE NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL

Subcommittee C recommended that further study be made of the tentative method for the determination of water-soluble nitrogen precipitable by 40 per cent alchol in conjunction with the method for eggs.

Preliminary observation shows that the addition of 5 cc. of 0.01 N acetic acid per gram of alimentary paste yields a clear filtrate, but the addition of 15 cc. of the sodium chloride solution (28 grams of sodium chloride made up to 300 cc. with water) to 100 cc. of the filtrate causes a copious precipitate which dissolves when made to 200 cc. with 95 per cent alcohol.

Before a method is ready for collaborative study, it is necessary to prove definitely whether or not it will yield satisfactory results when applied to products of known composition whenever this is possible. The solubility of proteins is enormously influenced by the H-ion concentration, the presence or absence of salts, the presence or absence of alcohol, and the temperature. To date no time has been available to make the necessary nitrogen determinations.

It is recommended[1] that the work on methods for the determination of water-soluble nitrogen precipitable by 40 per cent alcohol be continued.

REPORT ON CRUDE FIBER IN ALIMENTARY PASTE, BREAD, AND BAKED PRODUCTS

By R. G. CAPEN (Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

The method for the determination of crude fiber in alimentary paste, bread, and baked products was further studied. The official method under Grain and Stock Feeds[2] was compared with a modification of the Italian method (L. Bellucci).[3] This method (boiling the substance one hour with glycerol plus 2 per cent sulfuric acid) gave results comparable to those obtained by the official A.O.A.C. method, but no advantages were observed. Therefore the associate referee limited the study to the official A.O.A.C. method.

The quantity of the sample of material was varied, 2, 5, 10, and 15

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 63 (1933)
[2] *Methods of Analysis, A.O.A.C.*, 1930, 280.
[3] *Ann. chim. applicata*, 22, 22 (1932).

grams of material being used. The large samples were very difficult to filter owing to the gumminess of the residue and did not give satisfactory results. Therefore the 2 gram sample was recommended for collaborative work.

Samples of bread, macaroni, and cake—air dried—were sent to four collaborators. The results follow:

COLLABORATOR	L. H. BAILEY per cent	V. E. MUNSEY per cent	M. L. OFFUTT per cent	R. G. CAPEN per cent
Bread	0.36	0.33	0.35	0.43
	0.38	0.32	0.30	0.44
Cake	0.18	0.27	0.42	0.30
	0.18	0.27	0.39	0.36
Macaroni	0.22	0.27	0.35	0.29
	0.22	0.29	0.32	0.31

It is therefore recommended[1]—

(1) That the A.O.A.C. tentative method for the determination of crude fiber in bread and alimentary paste (official, first action) be made official (final action).

(2) That methods for the determination of crude fiber in baked products be further studied.

REPORT ON MILK SOLIDS IN MILK BREAD

By ARNOLD JOHNSON (1403 Eutaw Place, Baltimore, Md.), *Associate Referee.*

In determining the quantity of milk solids in bread, several methods were tried. Use was first made of an observation reported to the associate referee that milk powder, which had been previously dried at 100°C. at 0.5 cm. pressure for 5 hours, lost 17 per cent of its weight when heated at 130° at atmospheric pressure for 3 hours, whereas cereal products lost only about 0.5 per cent of their weight on being reheated in this manner. Considerable work was done on this method, but the results were so erratic that it was finally discarded. Moreover, as bread is likely to contain only from 4 to 8 per cent of milk solids, the additional loss in weight on holding at 130°C. (by which the milk solids in the bread would be calculated) appeared to be too small to warrant further work.

Attempts were next made to determine several of the milk constituents with the idea that since these constituents were present in fairly constant quantities in milk, their determination would allow the calculation of the total milk present. A procedure suggested by Levites[1] was used for the

determination of casein. Ten grams of the dried bread was mixed with 100 grams of a mixture of 79 grams of pyridine and 36 grams of water. The insoluble material was allowed to settle, and 25 cc. of the extract was pipetted off. An additional 50 cc. of pyridine was then added to the 25 cc. of extract. Under these conditions the casein in the extract should have yielded an abundant precipitate. An alternative method of precipitating the casein consisted in acidifying 25 cc. of the pyridine extract with hydrochloric acid to pH 4.6–4.7 and then determining the precipitated casein. When some types of milk products were used in the bread, this method appeared to work fairly well, but in others difficulty was experienced both with obtaining the precipitate and with determining the casein in it. Further work is being done with this method.

The work on the estimation of milk in bread also included the determination of the lactose. From the value obtained for this constituent, the milk solids in the bread were calculated. Ten grams of the dried bread was extracted with water. Sugars other than lactose were fermented away with yeast. The yeast, protein, and suspended matter were then precipitated by the phosphotungstate method of Rumsay,[1] and the lactose was determined. The reduction of the Fehling solution was conducted by the Quisumbing and Thomas method,[2] and the reduced copper was determined by the method of Shaffer and Hartmann.[3] The results by this method appear to be promising, and work is being continued.

The bread used in making all the tests was dried and ground. It was taken from inside the loaf as the bread in the crust was not considered to be representative material, particularly because the high heat on the surface probably changed the milk constituents so as to make their determination difficult.

It is recommended that the work be continued, especially that phase dealing with the determination of lactose in bread and the calculation in the milk solids therefrom.

REPORT ON RYE IN FLOUR MIXTURES

By J. H. BORNMANN (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

The detection and estimation of rye flour in mixtures of wheat and rye flours has been a subject of study by food chemists for many years. It is a comparatively simple matter to determine whether any given pure flour is wheat or rye, but the detection and estimation of small amounts of either in mixtures is difficult. The detection of adulteration is also complicated by the fact that a "pure" flour is not necessarily pure, since the

[1] Am. Inst. of Baking Bull. 8 (1922).
[2] *J. Am. Chem. Soc.*, 43, 1503 (1921).
[3] *J. Biol. Chem.*, 45, 349 (1921).

standards for wheat permit 7 per cent of rye, and the standards for rye permit 10 per cent of wheat. Naturally the flour milled from such grain will contain an appreciable amount of wheat flour, or of rye flour, as the case may be. The problem of the chemist then becomes the differentiation between accidental and intentional addition.

A review of the literature showed that the question has been attacked from a number of angles, in most cases with rather indifferent success. The stumbling block has usually been the similarity of wheat and rye. It may be of interest to sketch briefly some of the methods that have been proposed.

The Bamihl test, as modified by A. L. Winton[1] has long been known and used chiefly as a test for wheat flour in rye flour. It is simple and requires little time, but it is not applicable in the case of small quantities of rye in wheat flour.

The chloroform test[2] has been in use for over 40 years, and appears to be of considerable value after the analyst has had sufficient experience in its use. This test is performed as follows:

10 grams of flour is put into a test tube and treated with 20 cc. of chloroform· The tube is stoppered and shaken thoroughly, after which it is allowed to stand long enough for the heavy portion to settle to the bottom and the light portion to rise to the top. The aleurone grains, being the heaviest, are deposited on the bottom. The aleurone layer of rye is bluish or greenish, while that of wheat is yellowish or brownish. Comparison of a known pure wheat flour with a pure rye flour readily shows a marked difference in color, even to the inexperienced analyst. The addition of 10 per cent of a light colored rye flour to wheat flour can be detected by this test. A dark rye flour can be detected in smaller amounts, though a dark flour would probably not be used for blending. This method will probably be of value chiefly in the routine examination of a large number of flour samples, as by its use the pure wheat flours may be eliminated. In the examination of self-rising flour and flour containing added phosphate, it is necessary to remove added salts by a preliminary treatment with carbon tetrachloride. The flour rises in carbon tetrachloride while the salts settle out and may be removed by means of a separatory funnel. The chloroform test is not applicable to baked products.

J. Abel[3] proposed a method based on the washing out of gluten from the rye flour. He found that the addition of rye flour to wheat flour lowers the gluten recovery disproportionately and prevents it completely if enough flour is present. He found the point of "gluten failure" to be at about 40 per cent wheat flour. He suggests that this point may vary with different samples of flour. Results were obtained showing the decrease in dry gluten for each increase of 10 per cent of rye flour. This decrease is but 0.9 per cent for the interval 0–10 per cent rye flour and gradually increases with increasing percentages of rye flour.

The method appears to be tedious and of doubtful value as the natural

[1] U. S. Dept. Agr. Bull. 122, 217.
[2] F. Benecke. *Landw. Ver. Sta.*, 36, 337 (1889).
[3] *Z. Unters. Nahr. Genussm.*, 39, 44 (1920).

variation in gluten content of different wheat flours is probably greater than the variation due to 10 per cent added rye.

R. Fanto[1] attempted to determine small quantities of rye flour in wheat flour by the measurement of the viscosity of an aqueous extract. The extract is prepared by shaking 10 grams of flour with 100 cc. of water for 2½ hours. It is then centrifugalized for 1 hour, filtered, cooled to 17°C., and the time of outflow from a 50 cc. pipet is determined. The pipet is standardized with water at the same temperature. The pipet used had an outflow time of 51 seconds for water. The highest value found for wheat flours was 72 seconds, while the lowest value for rye flour was 133.5 seconds. With a given wheat and rye flour it is possible to detect the addition of 2½ per cent of rye to the wheat. In dealing with an unknown flour the limiting values are too uncertain for the detection of such small quantities.

Geilinger and Schweizer,[2] after discussing various methods, arrive at the conclusion that the method of Gury[3] gives the best results. In this method a 2 gram sample of flour is treated with water, centrifugalized in a graduated tube, and the height of the sediment is read. This procedure is repeated with 95 per cent alcohol. The sediment height for wheat flour is greater in the case of alcohol, whereas for rye the reverse is true. The author does not claim any great accuracy for this method.

R. Strohecker[4] attempted to develop a method based on the refractive index, and also on the specific conductivity, of an aqueous extract of flour. In the case of mixtures of wheat and rye flours the percentages calculated from the refractive index agreed closely with the theoretical, probably because the values for the components were known. In the case of mixtures of unknown flours it would be necessary to use average values, in which case the accuracy could not be great.

A method proposed by König and Bartschat[5] is based on the fact that a saturated aqueous solution of calcium sulfate dissolves about one-fourth of the total protein of wheat, whereas it dissolves about one-half of the total protein of rye. The essential part of this method is as follows: Total protein is determined in 2 grams of the flour; 10 grams of flour is put into a 500 cc. Stohmann bulb, 50 cc. of saturated calcium sulfate solution is added, and the mixture is shaken to a uniform suspension. The bulb is then filled to the mark with saturated calcium sulfate solution, and shaken for 1 hour in a shaking machine at 40 revolutions per minute. The mixture is filtered at once on a folded filter, and protein is determined in 200 cc. of the clear filtrate. From these results the percentage of total protein soluble in saturated calcium sulfate water is calculated. A chart is given which shows the percentage of wheat and rye flours corresponding to any given

[1] *Z. Unters. Nahr. Genussm.*, **28**, 79–83 (1914).
[2] *Mitt. Lebensm. Hyg.*, **16**, 95 (1925).
[3] *Ibid.*, **4**, 113 (1913).
[4] *Z. Unters. Nahr. Genussm.*, **47**, 90 (1924).
[5] *Ibid.*, **46**, 321 (1923).

percentage of total protein soluble in the calcium sulfate water. The authors obtained good results on known mixtures of wheat and rye provided the acidity was not greater than 5 cc. normal alkali per 100 grams, and provided also that the wheat was not exclusively hard wheat. The method is not suitable for baked products.

J. Tillmans[1] and his students have discovered a new carbohydrate in rye flour. This substance appears to be trifructoseanhydride. Rye contains about 2 per cent of this polysaccharide, whereas wheat contains only about one-tenth as much. Corn, rice, oats, and barley do not contain it. A qualitative test for rye flour in wheat based on the detection of trifructoseanhydride has been proposed by Tillmans as follows: 5 grams of flour is treated with 20 cc. of 70 per cent alcohol and shaken for 15 minutes. It is cooled for 10 minutes at $-3°C$. with frequent stirring. It is then centrifugalized for 5 minutes, poured off, and filtered. Ten cc. of clear filtrate is treated with 0.5 cc. of $N/1$ sodium hydroxide in 70 per cent alcohol. The extract from pure wheat flour treated as above gives at most a faint turbidity. Rye, if present, causes a marked turbidity, or even a precipitate. Tillmans states that 10 per cent of rye in wheat flour can easily be detected by this test. For the detection of smaller amounts of rye he recommends a quantitative method developed by his students.

The Tillmans method has been tested by other investigators with favorable results. Schweizer[2] reports that he found it the most satisfactory of the various methods considered by him. Beythien[3] also reports that he found it a reliable method. Kruisheer[4] has worked out a procedure for the quantitative determination of trifructoseanhydride upon which he estimates the rye content of flour mixtures. After hydrolyzing the sodium salt of trifructoseanhydride obtained according to the method of Tillmans, he first determines total reducing sugars. The fructose is then oxidized with hypoiodite and the residual reducing sugars are determined. This author states that rye flour contains 1.5–2.0 per cent of trifructose, while wheat flour contains but 0.1–0.3 per cent. The rye flour content is estimated from the trifructose found. The author states that the results are ± 10 per cent owing to the natural variations of rye and wheat.

R. Strohecker[5] has published the most recent contribution on the Tillmans method. After some preliminary work to determine how the difference between wheat and rye could be emphasized, and bearing in mind the desirability of having a method applicable to both flour and baked products, he formulated the following modification of the method:

Thoroughly mix 10 grams of flour or ground bread with 100 cc. of water, so that no lumps remain. (It may be advisable to use a mortar for this purpose. Albuminoids are precipitated |by the addition of 5 cc. of dialyzed ferric hydroxide.) After 10

[1] J. Tillmans, H. Holl, and L. Jariwala. *Z. Unters. Nahr. Genussm.*, **56**, 26 (1928).
[2] *Mitt. Lebensm. Hyg.*, **20**, 119 (1929).
[3] *Z. ges.* Mühlenw. **5**, 191 (1929).
[4] *Rec. trav. chim.*, **50**, p. 153.
[5] *Z. Unters. Lebensm.*, **63**, 514 (1932).

minutes filter through a folded filter into a 100 cc. glass-stoppered cylinder. If filtration is slow, replace the filter. After collecting exactly 25 cc. of filtrate dilute to the 90 cc. mark with 96 per cent alcohol, shake, and again make up to the 90 cc. mark. Allow to stand 10 minutes and filter through a folded filter into a 50 cc. glass-stoppered cylinder. Collect 45 cc. of filtrate and add, by means of a pipet, 5 cc. of 0.5 N - KOH in alcohol. Shake, and allow to stand 30 minutes. Filter with suction on a Gooch crucible and wash twice with 5 cc. of 96 per cent alcohol. Dissolve the precipitate in several small portions of hot water, catching the washings in a test tube placed in the filter flask. (The solution is poured back into the 50 cc. cylinder and diluted to 50 cc.) Pour into a 100 cc. volumetric flask and rinse the cylinder with 25 cc. of water, adding the rinsings to the solution. Add 5 cc. of concentrated hydrochloric acid and invert at 70°C. After neutralizing the acid and diluting to the mark, determine reducing sugars in the usual manner. The copper is weighed as cupric oxide, and the results are expressed as milligrams of cupric oxide per gram of dry substance in the flour or bread.

Strohecker's report includes a number of tables giving the reduction values found for about 60 samples of pure flour, mixed flour, and bread. These values range from 63.4 to 75.5 mg. for rye flour and from 3.4 to 11.9 mg. for wheat flour. Values found for bread vary from 48.1 mg. for pure rye bread to 5.0 mg. for pure wheat bread. It is pointed out that the values found for bread are lower than those of the flours from which the bread was made. This indicates that some trifructoseanhydride is lost in the baking process. The author states that the accuracy of this method is 5–10 per cent in the case of flour and about 10 per cent in the case of bread.

In the opinion of the associate referee the methods most worthy of further consideration are the chloroform test, the method of König and Bartschat, and the method of Tillmans as modified by Strohecker. The first two are useful only in the case of flour, while the third has the advantage of being applicable to bread. In the examination of flour the three methods may be of value to supplement each other.

MICROSCOPICAL METHODS

A few words may be said regarding microscopical methods. Rye bran may readily be distinguished from wheat bran because the cross cells of the former are thickened at the ends, and the cell walls of the latter are much more prominently beaded. The hairs are also distinguishable, but as they are not plentiful on rye this fact is of minor importance. A quantitative method based on the counting of bran particles can have but little value because there is no fixed relation between flour and bran. A rye flour which is white enough for blending with wheat flour will contain a rather small amount of bran. The bran particles may be secured in concentrated form by using the portion of flour which rises to the top in the chloroform test. This is examined microscopically after boiling with dilute sulfuric acid to hydrolyze the starch.

Berliner and Koopmann[1] state that rye contains a pectin-like substance which swells when the flour is moistened. They make use of this fact by rubbing the flour with lampblack in a drop of water on a microscope slide. As the rye flour particles swell they become surrounded by a light-colored area and may be counted. Methods involving a microscopic count are tedious and of value chiefly as supplementary evidence.

It is recommended that the chloroform test, the method of König and Bartschat, and the Tillmans method be studied collaboratively.

ADDITIONAL REFERENCES

H. Freund. *Pharm. Zentralhalle,* 55, 411.
K. Seidel. *Z. ges. Getreidew.,* 15, 125.
H. Kühl. *Ibid.,* 17, 122.
A. Giovanardi. *Ann. chim. Applicata,* 21, 296.
N. A. Trofimuk. *Z. Unters. Lebensm.,* 52, 311.
Lenz and Kraft. *Z. öffentl. Chem.,* 1909, p. 224.
Berliner and Koopmann. *Z. ges. Mühlenw.,* 1928, p. 42.

REPORT ON EXPERIMENTAL BAKING TESTS

By C. G. HARREL (Pillsbury Flour Mills, Minneapolis, Minn.), *Associate Referee*

Some definite progress has been made under a Fellowship of the American Association of Cereal Chemists held by P. P. Merritt and supervised by C. H. Bailey, M. J. Blish, and D. A. Coleman. The detailed reports have been published.[2] This Fellowship accomplished much toward mechanizing and methodizing the tentative baking method of the A.A.C.C. It is urged that in the installation of new equipment the suggestions offered in these reports be given serious consideration, so that progress towards uniformity may be made.

The conclusions, recommendations, and suggestions of this Research Fellowship have also been published.[3] It is recommended to the A.O.A.C. that the following directions, which are essentially the same as those given by Merritt, Blish and Sandstedt,[3] with the exceptions noted below, be adopted.

Absorption.—Absorption should be adjusted to suit each individual flour, with special care not to have doughs too slack or sticky. It is further suggested that all doughs be brought to a condition of uniform plasticity by the use of the Brabender or other similar apparatus. The associate referee differs decidedly in his recommendations as to the temperature limits permissible for doughs coming from the mixing operation and urges the A.O.A.C. to confine temperature limits to plus or minus 0.5°C.

[1] *Z. ges. Mühlenw.,* 5, 21 (1928).
[2] *Cereal Chem.,* 8, 265 (1931); 9, 175 (1932).
[3] *Ibid.,* 9, 235 (1932).

Yeast.—The associate referee also differs with the statement that the freshness of the yeast supply is not a critical factor. It is urged that the A.O.A.C. make use of definite tests to be assured of uniformity in their yeast supply. Further, if serious work is to be undertaken, that they confine themselves to a definite type of yeast.

Fermentation Bowls.—The Fellowship findings given on page 235 are strongly advised.

Punching Doughs.—The suggestions given under this heading, page 235, are deemed constructive and worthy of further study insofar as they apply to the tentative baking method.

Molding and Panning.—The suggestions given on pages 235 and 236 are heartily endorsed, and further work is urged along these lines.

Baking.—The recommendations given by Merritt, Blish, and Sandstedt in regard to more definite heating sources, to afford a better opportunity for differentiation of top crusts, cannot be over-emphasized. Their suggestion of the elimination of top heat is well founded and it is urged that this be complied with until further specifications have been formulated.

Baking Pans.—The associate referee recommends the findings of the Fellowship as set forth under this heading.

Loaf Volume Measurement.—The importance of this cannot be over emphasized, and it is urgently recommended that workers standardize their volumetric equipment as suggested in the report of the activities of the A.A.C.C. Baking Fellowship, page 236.

Supplementary Procedures.—Under present conditions of flour testing, the basic procedure as given in *Cereal Chem.*, 6, page 249, gives definite characteristics in the baked bread. These characteristics are further confirmed in the great majority of cases by the use of one of the supplementary procedures given in *Cereal Chem.*, 6, page 251.

It is a well established fact in commercial testing that the basic procedure is not used as frequently as the supplementary procedures. The data and information to be obtained make this necessarily so. In the interest of uniformity, so that data may be more readily comparable, it is distinctly urged that more definite standardization on these most useful supplementary or confirmatory procedures be undertaken, and the addition of further supplementary procedures be delayed until this standardization has shown definite progress.[1]

The paper, "The Rôle of Dairy Products in Baking," by Washington Platt, was published elsewhere.[2]

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 64 (1933).
[2] *Cereal Chem.*, 10, 213 (1933).

REPORT ON RHUBARB AND RHAPONTICUM

By Arno Viehoever[1] (Philadelphia College of Pharmacy and Science, Philadelphia, Pa.), *Associate Referee*

The value of rhubarb as a laxative is so well established that any substitution of an inferior product must be prevented. Unfortunately the botanical source is hard to control; the chemistry, in spite of much study, not completely solved; and the physiology, surprisingly, still somewhat obscure. Similar conditions prevail in the case of substitutes, sometimes placed in commerce, no doubt, in ignorance, sometimes out of greed for undue gain.

TAXONOMIC DATA

Rhubarb.—The area of distribution of the genus *rheum*, as W. Himmelbaur of Vienna, has pointed out (see Report in Budapest, 1928, during the International Congress on Medicinal plants) reaches from East Asia over the Himalaya-Middle Asia section and over the Northern Mountains (Balkan Range) and embraces about 40 species. Several of these species may actually yield the commercial Chinese drug. As rheum species may be readily crossed, hybrids are likely to be formed, even in the country of origin. The securing of absolutely reliable material from the natives has been very difficult.

Nevertheless it has been shown that rhizomes of the European medicinal rheum type with palmate leaves equal the rhizomes of the Chinese type, which appears to have the property of yielding massy orange yellow, strongly flavored rhizomes. They show a Borntraeger reaction (becoming red with alkali) and act biologically upon mice and men as does the genuine Chinese drug. Thus Himmelbaur in Austria and Tschirch in Switzerland encouraged the cultivation in Europe of good medicinal rhubarb, especially by vegetative means. Germany alone used more than 100,000 kg. of the drug according to an estimate in 1928.

Rhaponticum.—*Rheum rhaponticum* originally, in the 17th century, came from China by way of Siberia to the Balkan mountains, reaching Italy, Hungary and then about 1800 Germany, France and again England, where it had already been introduced 200 years previously. As a consequence of these shipments and reshipments, in the course of centuries, England cultivated mainly *Rheum palmatum* and *rhaponticum*, France, *rheum officinale* and *rhaponticum* and Austria of the prewar territory, *rheum rhaponticum.*

The frequent mixing of types and the growing of inferior rhizomes gave unsatisfactory results and the European types acquired a bad reputation. The food type of rhubarb proved altogether useless as a laxative. Rhaponticum, though its inferiority appears established, is still substituted for the usually more expensive Chinese rhubarb.

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[1] Presented by L. E. Warren.

MORPHOLOGICAL DATA

Differentiating characteristics, which are of limited value, are enumerated elsewhere in the tabulations.

CHEMICAL DATA

The nature of rheopurgarin in rhubarb deserves special attention in further studies. Additional data are expected from E. Siegrist's publication,[1] which appeared in 1932 in Basel. The following tests for rhaponticum appear promising:

Isolation of rhaponticin.—Exhaust 10 grams of powder by percolation with 60 per cent alcohol until 25–26 grams of percolate is obtained. Filter, if necessary, and evaporate on a water bath at 80°C. until the residue weighs about 7 grams. Add 10 cc. of ether, shake well and allow to stand. In about 4 hours a brownish crystalline deposit separates out.

In a mixture of 25 per cent rhubarb and 75 per cent rhaponticum the crystallization takes about 24 hours; with 75 per cent rhubarb and 25 per cent rhaponticum the crystalline deposit is only observed after longer standing. Various modifications for the rhaponticin isolation and identification are available, but the application of microanalytical methods deserves most attention.

Another test, which might be worked up as an alternative for the detection of rhaponticum in the official rhubarb, is carried out as follows:

Mount a little of the powdered drug in water and wash three times with water, which is then removed as completely as possible. Float a mixture of 10 parts of 50 per cent aqueous solution of potassium hydroxide and 10 drops of hydrogen peroxide (100 per cent by volume) on the moist powder and allow the preparation to stand for 30 minutes. Particles of the rhapontic powder appear to assume an intense blue color, apparently due to a granular precipitate. The particles of other rhubarbs, so far as examined, are never blue or granular.

This test also deserves further attention with special consideration given to the nature of the blue coloration and granulation. Fractional sublimation of both rhubarb and rhaponticum will, doubtless, give better results than will uncontrolled sublimation, yielding evidently more than one type of crystal in the sublimates of either powder. Chrysophanic acid, when sublimed, appears colorless in the pure state. The definite identification of the subliming constituents of both rhubarb and rhaponticum must be the next aim.

FLUORESCENCE

Considerable attention has been given, first abroad by Maheu[2] and then in this country, to the violet fluorescence of rhaponticum, even in high dilutions. The substance causing the fluorescence has not been isolated thus far. It was found that many papers (filter paper and toweling ma-

[1] Thesis for Pharm. Inst. of Univ. Basel, Switzerland (1930).
[2] *Bull. Sci. pharmacol.*, **35**, 278 (1928).

terial, etc.) gave a violet fluorescence similar to that of rhaponticum. The aqueous extracts, hot or cold, show a violet fluorescence, changing to whitish upon alkalinization, disappearing when filtered through charcoal. Ether-acetone extracts, containing the yellow pigment, showed a striking red fluorescence. The liquid extractions appeared brighter in color than the dried residue of the same liquids. Different intensities and shades of fluorescence were observed, depending upon whether the extracts were moist or dry. The orange fluorescence observed in many extracts is doubtless due to chrysophanic acid, as check experiments have proved.

Rhaponticin, apparently, shows no fluorescence. In this connection the observation of Petri and Cucco is interesting, as they found 150 fluorescing substances, as glucosides, phloroglucinol tannins, or plant pigments in 164 plants examined. Other workers have found resins (from conifers) and alkaloids (as atropine, hyoscyamine, cocaine and novocaine) fluorescent. The abundance of fluorescing substances found in plant products lowers the value of the property of fluorescence in detecting rhaponticum by this means in extracts. Of special interest is also Glaser's recent observation that increasing acidity in aesculetin derivatives removes fluorescence, that methyl aesculetin shows stronger fluorescence than aesculetin and that aesculin shows no fluorescence at all. The presence or absence of another group in anthraquinones, or the degree of acidity, may have a definite bearing upon the type and intensity of the fluorescence,—or even explain its obscurity or absence in certain cases.

PHYSIOLOGICAL DATA

As the tabulation of physiological differentiation indicates, comparatively large amounts of rhaponticum are necessary to cause a laxative effect; thus the consideration of inferiority for rhaponticum appears justified. The rhizomes from the palmate forms of rhubarb acted most nearly like the Chinese official rhubarb. The rhizomes of round-leafed types were without effect, although certain of their offsprings sometimes proved effective. Himmelbaur, reporting these findings, made no extensive experiments on man, using mainly white mice. Fuehner and Fernandez[1] tested the effect of hydroxyanthraquinones, speeding action of the intestines of white mice, and found on comparing seven hydroxyanthraquinone and four trihydroxy anthraquinone with rhubarb, rheopurgarin, rheum emodin and chrysophanol, that emodin and chrysophanol—even in large amounts—were inert. The action of rhubarb and its glucoside fraction rheopurgarin was definite.

MICROPHYSIOLOGICAL DATA

The glucosides of the anthraquinones and the oxymethylanthraquinones, as Tschirch[2] states, are slowly hydrolyzed as they pass through the

[1] *Arch. Exp. Path. Pharmakol.*, 124, 185 (1927).
[2] *J. suisse pharm.*, 36, 237 (1898).

intestine. He believes that the anthraglucosides pass in the intestines as well as in the plants in amorphous colloidal form and thus are pharmacologically more active.

Qualitative tests were made with aqueous extracts of rhubarb, rhaponticum and rhaponticin upon the crustacean daphnia with very gratifying results. In all cases peristaltic movement of the intestines, clearly visible under low-powered microscope, is increased, complete evacuation following within 4 minutes in the case of rhubarb under the conditions of experimentation. The application to quantitative work is under way; it has already been established for depressants and stimulants.

While progress has been made, further work should be done to obtain a satisfactory chemical method for the isolation and characterization of "rheopurgarin" and its physiological standardization as well. Further work should be done on the isolation and identification as well as the ready detection of the fluorescing substance that gives to rhaponticum the characteristic violet fluorescence. A simple, preferably microanalytical, method based upon the presence of rhaponticin should be perfected to permit quick and sure chemical differentiation of rhaponticum root. A chemical quantitative method, supplemented by a physiological method, for the evaluation of rhaponticum root should be worked out.

It is recommended[1] that work be continued and that the tests, as soon as perfected, be submitted to collaborators for confirmation or comment.

The methods, applicable to the differentiation of these root drugs, may be divided into morphological, histological, chemical, physico-chemical, and physiological.

DIFFERENTIATION
Morphological

Rhubarb	Rhaponticum
Cylindrical, club-like or almost round	Club-like or cylindrical and often bent
Plano-convex or flattened pieces	
Peeled pieces	Peeled pieces
Yellowish, hard and heavy	Brownish or darker yellow, rather light, smaller, with pith center

Histological

Pith rays curved	Pith rays straight

Transverse Section

Interior shows marbled appearance through orange red points or lines	Interior shows light yellow and brownish-red, star-shaped lines, reaching almost to the center

Chemical

Yields an orange brown extract with water	Yields a muddy looking yellow extract with water; the filtrate develops a brown sediment, partly due to rhaponticin.
The filtrate remains clear	
(The powder, its extracts, and solutions are colored red with alkalies)	(The powder, its extracts, and solutions are also colored red with alkalies)

Contains rheopurgarin, a combination of glucosides, related to emodin and rhein, with the glucoside chryso-phanein, yielding chrysophanic acid, and rheochrysidine, possibly identical with rhabarberone or iso-emodin	Rhaponticin Rheotanno glucoside Anthra glucoside, yielding rheonigrin
Rhein	Chrysophenol—methoxyl free chryso-
Rheinolic acid	phanic acid
Emodin monomethylether	Emodin monomethylether
Emodin and aloe-emodin	Emodin
Chrysophanic acid	Chrysophanic acid

Microchemical

Rhaponticin present; yields, upon hydrolysis, rhapontigenin and *d*-glucose

Sublimation

Yields dark colored needles, varying in size, attached to light yellow masses	Yields both large and small sized needles, either single or in bunches with star-shaped formation

Ultraviolet Radiation

Reaction obscure, dark gray to dull grayish brown fluorescence	Striking fluorescence, somewhat violet; the finer the powder, the brighter the color

Medullary Rays

Show very faint lavender fluorescence	Show a marked shimmery fluorescence

Alcohol Extract with Ether

Orange brown fluorescence	Purple brown fluorescence

Physiological*

Palmate-leaf forms	(per gram of mouse)
	gram
1. *Rheum chinense* (Commercial Drug)	
2. *Rheum palmatum hortulanorum* (Korneuburg)	0.0012
3. *Rheum palmatum proles* (Przewalski)	
4. *Rheum palmatum proles* (Tafelii)	0.002–.003
Round-leaf forms	
5. *Rheum palmatum proles* (Przewalski)	
6. *Rheum palmatum proles* (Tafelii)	0.003–.004
7. *Rheum rhaponticum*	0.004

* Quantity of drug necessary to cause laxation in 4 to 6 hours after Himmelbaur.

TUESDAY—AFTERNOON SESSION

Chairman: There being no announcement this afternoon, the first speaker is the Director of Scientific Work of the United States Department of Agriculture, and I have asked Secretary Skinner to introduce Doctor Woods to you.

Dr. Skinner: I do not know just why the President of our Association has asked me to introduce Doctor Woods. He probably needs no introduction. He may have had in mind the fact that at one time I was Doctor Woods' boss and now he is mine. I treated him very well, I thought. I am hoping he will treat me well. He has so far. Doctor Woods has had a very distinguished career. He came to the Department of Agriculture years ago and was instrumental in organizing a large part of the research work of the Bureau of Plant Industry. After serving some years in that capacity and in various capacities as administrative officer and research worker, he left the Department to take charge of a great work in Minnesota, where he added to his reputation both as a scientist and organizer. By invitation, in 1916, he became president of the University of Maryland and was with that institution for 10 years—a period of marked progress. He was then invited to become Scientific Director of the U. S. Department of Agriculture. In that position he has also made splendid progress. The research work of the Department has never in its history been on such a sound foundation as at the present time, and that is largely due to the scientific support given by Doctor Woods. This institution he knows well. The Association of Official Agricultural Chemists is appreciated by him, as he has told us on previous occasions. He comes here as no stranger but as one engaged in work in which all of us have a common interest. While not a chemist, he is on the border line of a chemist. He fully appreciates the relationship that must exist between the various scientists. It gives me great pleasure to ask Doctor Woods to address you.

ADDRESS BY DR. A. F. WOODS

This is my speech, ladies and gentlemen (holds up large batch of papers). I won't stop to read it all, but I shall pass it around. There is one here for each one of you, and I should like to have you study it because it furnishes the general basis for some of the things we must do to take care of the type of work we are doing, research work and other fundamental work that is being done in the Department of Agriculture and in the various State agencies that are working for the welfare of the people at large.

Never in my forty-two years of practical business connection with agricultural education and research has there been a time like this, a time

when there was such organized propaganda against the type of work we
are doing as exists today. It is led by people who ought to know better,
and who do know better, but who are motivated by the idea that taxes
must be reduced, regardless of how it is done. Now, we are all heartily
in favor of reducing taxes. We are taxed the same as everybody else and
we have to pay our share. We know that business can't develop, our work
can't receive support, schools can't run, departments can't be organized
and financed to do their work, unless there is money to do it with. We
want to study this whole question of whether too much money is being
expended in governmental work, whether any of it is being wasted,
whether this situation has been creeping up on us year by year at a rate
that we have hardly realized, or whether we are simply suffering from the
direct effects of that tremendous world cataclysm known as the World
War.

We try to trace the causes of these different reactions back to one thing
and another, and we find after a while some apparently definite and cer-
tain bases on which we can rest. One of them is the fact that three hundred
billion dollars' worth of purchasing power was destroyed in the countries
of the world during the World War. Three hundred billion dollars! Just
think of it! The entire wealth of the United States! Now, when you de-
stroy the purchasing power of all the countries you deal with and they
can't buy until you lend them money, you can see where we are. We lent
them ten billion dollars and during the period of spending it there was a
spurt of international trade, but now they haven't any more and can not
get more unless they can borrow it, and we are up against another situa-
tion where a readjustment must be worked out.

Now, I want you to study this chart (charts distributed). You will see
when you study it that the ordinary expenses of the Federal Government
run about one billion dollars. This estimate is based on official figures from
the United States Department of Commerce and is published by the Na-
tional Association of Manufacturers of the United States, who want the
facts and also want the facts distributed. It is the clearest picture I have
seen of what is happening and why it is happening. Of course, the next
thing is what are we going to do about it.

There are various organizations making studies of this situation. I
want to call your attention to one or two of the agencies you need to keep
in touch with. One of these is the Taylor Society of New York. It is a
society of economists and engineers who study business organizations from
every angle with the idea of trying to get the fundamental trends and
causes. A very interesting series of articles which points out fundamental
facts began in the monthly bulletin of this organization last month and,
so far as we can, we ought to get hold of that bulletin.

I want to read one or two excerpts from it. Concerning the dependence
of industry on the small wage earner, this society points out that "nearly

80 per cent of the total volume of goods purchased yearly in this country is bought by wage earners receiving less than $5,000 a year, 67 per cent by those receiving less than $3,000, 36 per cent by those getting between $1,000 and $2,000, and about 18 per cent by those earning less than $1,000. At the same time the decline in real payrolls—that is, payrolls expressed in terms of the amount of goods they will buy—has been 50 per cent, a decrease of more than five billion dollars in the purchasing power of some 8,500,000 factory workers in the United States."

You will see that our big problem is not production. We have the business of production pretty well worked out. There is much to do. We can make great advances, and we are making them. The machinery of production in industry and in agriculture is able to produce all that people can buy. The problem now is to find out how to distribute the wealth so that those who do the buying can buy—that is, those who get small wages and salaries. That problem is being studied very carefully now by the Taylor Society and business organizations of one kind and another, and the Associated Press and other news agencies are becoming interested in distributing this kind of information.

We need to stabilize production and to set up economic machinery that will enable us to go on with the production of wealth and then distribute it as widely, as fairly, and as equitably as possible. We must devote our time to other than the scientific problems we investigate. We must stop for a moment and give our careful attention to these other questions. We must be familiar with the facts—those great social facts which underlie business. It is our advice and our cooperation that is going to help direct various business agencies along right lines, and we must see that sound, safe and accurate scientific information gets to them and that the economic information that is worked up for us is based upon sound scientific principles.

My speech today is mainly that of a warning. You are facing today the most difficult situation for financial support of your work and for its continuance as a part of the social program that you have ever faced. We are facing the problem of financing research work, of maintaining the salaries of our employees, and of enabling the people to pay the taxes and to buy the kind of work we are able to sell. I think it is quite as important for us to give some thought to these problems and to familiarize ourselves with the facts as it is to devote our entire attention to our technical problems. The technical problems are important. They are more important than they ever were. There is more demand for their solution, and the work will continue if people can continue to pay for it.

I am not at all pessimistic. I picked up some of the latest business prophecies and they tell me that prosperity is just around the corner. Fortunately, it is around the corner we have just turned, and we are headed up; while we shall go slowly, we shall build with a greater sound-

ness than we built before. We are going to study the whole program. We are not going to let minorities here and there come in and take out millions and billions of dollars for this and that, unless it is something worth while. We are going to see that our business set-up is so adjusted that we can continue to grow without these great ups and downs, these severe depressions and great booms which always burst. *Human welfare* is our slogan from now on.

No report on beers, wines and distilled liquors was given by the associate referee.

REPORT ON SPECIFIC GRAVITY AND ALCOHOL

By Alfred W. Hanson (U. S. Food and Drug Administration, Minneapolis, Minn.), *Referee*

The alcohol tables given in the latest edition of *Methods of Analysis* cover the range of 0–25 per cent, by volume, at the official temperatures of 25/25° C., 20/20° C., and 15.6/15.6° C. If a larger quantity of alcohol is present in a sample, it can easily be diluted so as to come within the range of the tables. As stated in the footnotes accompanying the tables, the difference between the true and apparent specific gravities are negligible for this quantity of alcohol. While it is possible to increase the range of the tables, no such change is contemplated at the present time.

The method for the determination of specific gravity has been rewritten so as to conform to the new temperature ranges. The procedure has been divided into two parts and corresponds in this respect to the method for specific gravity of oils.[1]

The following method is submitted for consideration by the Association.

STANDARDIZATION OF PYCNOMETER

Carefully clean, dry, and weigh the pycnometer. Cool a flask of recently boiled, distilled water to a temperature about 4°C. below the temperature at which the determination is to be made. First rinse the pycnometer with this solution, then fill, avoiding air bubbles, and allow it to come to the desired temperature. (A constant temperature bath may be used for this purpose.) If a stoppered pycnometer is used, allow it to come to the desired temperature in a constant temperature bath and make the necessary volume adjustment before it is removed. Wipe it dry and weigh immediately. If the pycnometer is provided with a thermometer and perforated cap, wipe it dry when the solution is about 2°C. below the required temperature. Make the volume adjustment, cap, and dry it as the mercury reaches the temperature. Weigh the pycnometer immediately and record the weight of the pycnometer and water for future reference. The weight of water = W in the formula.

[1] *Methods of Analysis, A.O.A.C.,* 1930, 314.

Cool the flask containing the sample at about 4°C. below the required temperature. (A refrigerator or water bath may be used for the purpose.) Rinse the standardized pycnometer with the solution, then fill it, avoiding air bubbles. Proceed carefully with the temperature and volume adjustments as described in the standardization. It is desirable to have the temperature and time intervals uniform. Dry and weigh the pycnometer after the volume adjustment has been made at the required temperature. Determine the weight of the contained liquid by difference. Designate this weight as S in the formula. Calculate the specific gravity as follows:

$$\frac{S\ 15.56°C.}{W\ 15.56°C.}, \quad \frac{S\ 20°C.}{W\ 20°C.}, \quad \frac{S\ 25°C.}{W\ 25°C.}.$$ (For alcohol tables see pp. 532–537. The sample

should be diluted so as to bring the quantity of alcohol below 25 per cent by volume.)

REPORT ON VINEGAR

By A. M. HENRY (U. S. Food and Drug Administration, Philadelphia, Pa.), *Referee*

GLYCEROL

The investigational study reported last year indicated that diphenylamine as an inside indicator could be substituted for potassium ferricyanide as an outside indicator.

TABLE 1

Collaborative results (glycerol)

COLLABORATOR	GRAMS PER 100 CC. OF GLYCEROL	
	OFFICIAL METHOD POTASSIUM FERRICYANIDE INDICATOR	PROPOSED METHOD DIPHENYLAMINE INDICATOR
S. Alfend	0.293	0.293
	0.293	0.293
A. G. Buell	0.291	0.291
	0.292	0.290
S. L. Crawford	0.295	0.295
	0.295	0.296
R. L. Horst	0.294	0.294
	0.296	0.298
J. F. Laudig	0.292	0.292
	0.289	0.293
C. D. Shiffman	0.296	0.295
H. Shuman	0.293	0.293
Average	0.2933	0.2936
Variation	0.007	0.008

A solution containing 0.293 gram per 100 cc. of glycerol was sent to collaborators with directions:

(1) To pipet 25 cc. of the solution into a 250 cc. volumetric flask and determine glycerol as directed in paragraphs 2–5 inclusive, p. 360, *Methods of Analysis, A.O.A.C.*, 1930; and (2) using this oxidized glycerol solution to determine the glycerol as directed in last year's report.[1]

The results submitted by the collaborators are given in Table 1.

COMMENTS BY COLLABORATORS

S. Alfend.—I much prefer the diphenylamine titration to the outside indicator method. It is far more rapid and the end point is more easily detected. I think the directions should specify that when the blue-gray color is reached the titration should proceed slowly, for the violet color sometimes develops slowly, particularly with the oxidized solution, and may require 10–15 seconds to deepen sufficiently to be recognized.

A. G. Buell.—I prefer the use of the inside indicator. It saves considerable time in titrating.

S. L. Crawford.—The results using the two methods agree closely, but we prefer the diphenylamine indicator, because it is more convenient to use than the external indicator and spot plate.

R. L. Horst.—I much prefer the new method, as it gives a much better end point than is obtained by the official method with the outside indicator. Your formula, also, is much easier to handle.

J. F. Laudig.—The use of diphenylamine as an inside indicator is preferred.

C. D. Shiffman.—I prefer the inside indicator. I believe that the blue-gray color just before the violet is slightly closer to the outside indicator than the violet.

In view of the close agreement of the collaborators on the two methods (Table 1), the new method is recommended for adoption.

TOTAL AND SOLUBLE ASH AND PHOSPHORIC ACID

The effect of the addition of sucrose and other substances was studied by H. Shuman, U. S. Food and Drug Administration, with regard to reducing the time and temperature of ashing.

The substances tried, shown in Table 2, were dissolved in the vinegar, which was dried on the steam bath and then charred over a burner before being placed in the muffle. Five grams of substance was used in order to test its "swelling" properties and its ability to give a porous charred mass. The charred vinegars were ashed without moistening and temperatures were kept at lowest red heat (500° C.).

It is readily seen that the sugars are the better materials, and of these none offers any advantages over sucrose. Furthermore, sucrose is obtainable in a very pure condition (the ash in the sucrose used in these determinations was 0.0002 gram on a 5 gram sample). It swells readily and voluminously, giving the desired porosity.

Subsequent work on the use of sucrose showed that it is not desirable to use as much as 5 grams. Experiments with from 1 to 5 grams show that 2 grams gives the desired porosity and avoids excessive carbonaceous ma-

[1] *This Journal*, 15, 536 (1932).

TABLE 2

Sucrose and other substances as ashing aids

SUBSTANCE	QUANTITY ADDED	TIME OF ASHING	AMOUNT OF SWELLING OF CHARRED MASS IN 100 CC. PT. DISH	ASH	METHOD OF TREATMENT
	grams	*hours*		*per cent*	
Blank (Nothing added)	—	4	Very little swelling	0.339	Ash—incomplete after 3 hours (dry ashing). Material moistened once to complete ashing
1 gram sucrose	1	3	Swelled to ½ volume of dish	0.337	Complete after 3 hours at 500° C. (dry ashing). Fine carbonaceous particles present
5 grams sucrose	5	2	Swelled to ⅙ volume of dish	0.337	Completely ashed in 2 hours at 500° C.
Lactose	5	3	Swelled to ¼ volume of dish	0.342	Completely ashed in 3 hours at 500° C.
Dextrose	5	3	Swelled to ¾ volume of dish	0.360	Incompletely ashed after 3 hours at 500° C. Caked carbonaceous material present—further ashing abandoned
Mannite	5	3	Swelled to ¼ volume of dish	0.355	Incompletely ashed after 3 hours. (1 hour at 500° C. and 2 hours at 550° C.). Caked carbonaceous material present—further ashing abandoned
Citric Acid	5	3	Swelled to less than ¼ volume of dish	0.350	Same as for Mannite.
Tartaric Acid	5	—	Swelled to less than ¼ volume of dish	0.357	Same as for Mannite

terial. On the other hand, *one* gram of sucrose does not completely eliminate the tendency to cake or form dense carbonaceous residues in some samples.

In order to investigate the effect of different ashing temperatures on

TABLE 3

Effect of temperature and sucrose on the composition of ash of vinegar

ASHING TEMPERATURE	SUCROSE ADDED	ASH	SOLUBLE ASH	INSOLUBLE ASH	ALKALINITY OF THE SOLUBLE ASH	SOLUBLE PHOSPHORIC ACID	INSOLUBLE PHOSPHORIC ACID	TOTAL PHOSPHORIC ACID	PER CENT SOLUBLE PHOSPHORIC ACID OF, TOTAL	PER CENT INSOLUBLE PHOSPHORIC ACID OF TOTAL	RATIO: INSOLUBLE P_2O_5 TO SOLUBLE P_2O_5
°C	grams	per cent	per cent	per cent	cc.	mg.	mg.	per cent	per cent	per cent	per cent
500	None	0.314	0.048	0.266	2.96	1.95	11.45	13.40	15	85	5.87
550	"	0.302	0.044	0.258	3.10	1.99	11.29	13.28	15	85	5.67
600	"	0.303	0.044	0.259	3.05	3.32	9.74	13.06	25	75	2.93
					With Sucrose						
500	1	0.303	0.049	0.254	2.98	2.05	11.12	13.17	16	84	5.42
	2	0.314	0.052	0.262	2.98	2.06	Lost	—	—	—	—
	5	0.314	0.046	0.268	3.07	2.58	10.82	13.40	19	81	4.08
550	2	0.304	0.043	0.261	3.08	2.13	11.25	13.38	16	84	5.28
600	2	0.303	0.044	0.259	3.04	3.47	9.61	13.08	27	73	2.77

the ash and ash constituents (with particular reference to soluble and insoluble P_2O_5, and also the effect, if any, of the addition of sugar, samples of a cider vinegar were ashed at 500, 550, and 600° C., with and without the addition of sucrose, as shown in Table 3.

This work indicates that the effect of temperature may be disregarded, within the range used, for all determinations except soluble and insoluble P_2O_5. With these the effect is marked. This is especially shown in the ratios of insoluble to soluble P_2O_5. The total P_2O_5 remains, however, little affected.

The determinations to which sucrose has been added correspond closely to those determinations at the respective temperatures without added sugar, except in the one case in which 5 grams was used. Here again the effect is in the alteration of the ratio of insoluble to soluble P_2O_5, the total P_2O_5 remaining unchanged.

These results are in full agreement with those obtained by the former referee.[1] They show that temperature limits, within which a vinegar can be safely ashed without alteration of the insoluble-soluble P_2O_5 ratio, are narrow. The highest temperature permissible is 550° C. (dull red heat). Ashing at 500° C., which is the faintest red heat, or below this is impractical, as ashing proceeds very slowly for many vinegars and numerous moistenings are necessary. This condition indicates that unless the temperature is kept strictly at dull red heat (550° C.), or below, the insoluble-soluble P_2O_5 ratio is not reliable.

RECOMMENDATIONS[2]

It is recommended—

(1) That methods for the determination of total and soluble ash be further studied, with particular attention given to the use of sucrose or other substances for reducing the time of heating and to the temperature of ashing.

(2) That the methods for the determination of phosphoric acid be further studied in connection with the studies on ash.

(3) That the official method for the determination of total solids be studied, especially with reference to its application to vinegars high in solids, such as malt vinegar.

(4) That the proposed change in the glycerol method, which includes the substitution of diphenylamine as an inside indicator for potassium ferricyanide as an outside indicator, and the simplified method of calculating glycerol be adopted as official (first action).

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[1] *This Journal*, 10, 492 (1927); 11, 502 (1928).
[2] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 64 (1933).

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

By JOHN B. WILSON (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The work on flavors and non-alcoholic beverages was confined to a collaborative study of the two methods developed in the Bureau of Industrial Alcohol for the determination of essential oil in extracts and toilet preparations.

Extracts of almond, anise, lemon, nutmeg, orange, peppermint, rose, rosemary, spearmint, thyme, wintergreen and imitation wintergreen (methyl salicylate) were prepared by the referee and submitted to nine collaborators to be analyzed by Method I given in last year's report.[1] Extracts of cinnamon and clove were also prepared for analysis by Method II, also given in that report.

The results obtained are given in the accompanying table. Those obtained by collaborators familiar with the methods warrant the adoption of Method I as official for extracts of anise, lemon, nutmeg, orange, rosemary, thyme, wintergreen and methyl salicylate, and Method II for cinnamon and clove extracts.

It is believed that the erratic results obtained by some collaborators not familiar with the method, may be due to the use of unstandardized Babcock bottles or to the method of measuring the solvent. At the Bureau of Industrial Alcohol standardized bottles are used and the solvent is measured by means of a long 10 cc. buret. It was found that when this was done the results obtained were within 0.2 per cent of the quantity of oil present. Unfortunately, all collaborators were not informed of this method of measuring the solvent before they made the analysis, but a note to this effect will be incorporated into the method for next year's collaborators.

It has been suggested that the low results obtained on extract of rose are due to adulteration of the oil used. An effort will be made to obtain a sample of rose oil not open to this objection for collaborative work next year.

RECOMMENDATIONS[2]

It is recommended—

(1) That Methods I and II for the determination of essential oil in extracts and toilet preparations, given in the referee's report for 1931, be amended to specify the use of a 10 cc. buret for measuring the solvent added and of standardized Babcock bottles for reading the quantity of oil.

(2) That Method I be made official (first action) for extracts of anise,

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Determination of Essential Oil
(Results expressed in percentage)

COLLABORATOR		METHOD I												METHOD II	
		ALMOND	ANISE	LEMON	NUTMEG	ORANGE	PEPPERMINT	ROSE	ROSEMARY	SPEARMINT	THYME	WINTERGREEN	IMITATION WINTERGREEN	CINNAMON	CLOVE
W.O.W.	F. & D. Adm.	0.5	3.6	4.9	2.2	4.9	3.8	0.8	2.1	2.8	0.6	3.1	3.1	2.6	2.5
		0.5	3.6	5.0	2.2	4.9	3.9	0.8	2.1	2.8	0.7	3.0	3.0	2.6	2.5
J.I.P.	F. & D. Adm.	0.6	2.4¹	5.0	2.3	5.0	3.4	0.6	2.0	2.6	0.7	3.0	3.0	3.0	2.8
		0.8	2.5¹	5.2	2.4	5.0	3.4	0.8	2.2	2.6	0.8	3.0	3.0	3.0	2.8
L.B.	Bur. Industrial Alcohol		3.6	5.0	2.0	4.8	3.6	0.6	2.0	2.6	0.6	3.0	3.0	2.4	2.6
P.V.	Bur. Industrial Alcohol		3.4	4.8	1.9	4.8	3.4	0.8	2.0	2.6	0.4	3.0	3.0	2.4	2.6
W.H.F.	Bur. Industrial Alcohol		3.5	4.7	2.0	4.8	3.4	0.4	2.0	2.6	0.6	3.4	3.0	2.5	2.6
A.C.B.	Bur. Industrial Alcohol		3.6	4.8	2.0	4.9	3.6	0.4	2.0	2.6	0.6	3.0	3.0	2.6	2.6
J.W.	Kohnstamm & Co.		3.1	4.7	1.7	4.4	2.8	0.2¹	1.9	2.2	0.2¹		2.8	2.6	1.2¹
			3.3	4.4	1.9	4.6	3.0	0.0¹	1.4	2.3	0.2¹		2.7		
H.G.L.	Pease Laboratories		3.6	5.0	2.0	4.8	3.6	0.8	1.6	2.8	0.8	2.6	2.5	2.0	2.7
			3.6	4.9	2.0	4.8	3.4	0.8	1.8	2.7	0.8	2.8	2.5	1.8	2.7
R.L.R.	Pease Laboratories		3.5	5.0	2.2	4.8	3.4	0.4	1.8	2.6	0.6	3.0	2.9		
			3.4	5.2	2.2	4.8	3.4	0.4	1.9	2.6	0.6	3.0	3.0		
	Maximum	0.8	3.6	5.2	2.4	5.0	3.9	0.8	2.2	2.8	0.8	3.4	3.1	3.0	2.8
	Minimum	0.5	2.4	4.4	1.7	4.4	2.8	0.0	1.4	2.2	0.2	2.6	2.5	1.8	1.2
	Average	0.6	3.5	4.9	2.0	4.8	3.4	0.6	1.9	2.6	0.6	3.0	2.9	2.8	2.6
Oil Present		1.0	3.5	5.0	2.0	5.0	3.0	1.0	2.0	3.0	0.5	3.0	3.0	2.5	2.5

¹ Omitted from the average.

lemon, nutmeg, orange, rosemary, thyme, wintergreen and methyl salicylate.

(3) That Method II be made official (first action) for extracts of cinnamon and cloves.

(4) That Methods I and II be subjected to further collaborative work, with special study given to extracts not included in the above recommendations.

REPORT ON MEAT AND MEAT PRODUCTS

By R. H. KERR (Bureau of Animal Industry,
Washington, D. C.), *Referee*

Methods for the determination of salt in meat were studied. While simple in theory, this determination has sources of error which trouble the chemists that are not familiar with it. It is considered advisable, therefore, to include a method for salt in the chapter on meat and meat products.

Three samples were prepared and distributed. One of these, designated Sample A, consisted of a mixture of smoked and cooked sausages collected from various sources. Another, designated Sample B, consisted of a similar mixture of smoked and cooked sausages to which approximately 2 per cent common salt was added. The third, designated Sample C, consisted of cooked ham. All the samples were finely comminuted and thoroughly mixed, treated with a preservative to prevent decomposition, and packed in bottles with the stoppers sealed to prevent loss of moisture.

Collaborators were requested to determine salt according to each of the four following methods:

Method 1.—Moisten 2½–3 grams of the finely comminuted and thoroughly mixed sample in a platinum dish with 20 cc. of 5 per cent sodium carbonate solution, evaporate to dryness, and ignite at a temperature not exceeding a dull redness. Extract with hot water, filter, and wash. Return the residue to the platinum dish and ignite to an ash; dissolve in dilute nitric acid (1+4), filter to free from any insoluble residue, wash thoroughly, and add this solution to the water extract.

Determine chlorine in the combined filtrate and washings by the A.O.A.C. method for chlorine in plants.[1]

Method 2.—Weigh 2½–3 grams of the finely comminuted and thoroughly mixed sample into a 300 cc. round, flat-bottomed, boiling flask, preferably of Pyrex glass, add sufficient 0.5 *N* silver nitrate solution (5 cc. or more) to precipitate all chlorine and leave an excess. Wet the meat with the solution, then add 15 cc. of concentrated nitric acid, heat to boiling and boil until the meat is thoroughly disintegrated (approximately 10 minutes). Add 5 per cent potassium permanganate solution in successive small portions, boiling until the color of permanganate disappears after each

[1] *Methods of Analysis, A.O.A.C.*, 1930, 111, 34 and 35.

Collaborative results (expressed in percentage)

ANALYST	SAMPLE A—SMOKED AND COOKED SAUSAGE						SAMPLE B—MIXED SMOKED AND COOKED SAUSAGE WITH ADDED SALT						SAMPLE C—CURED AND COOKED HAM					
	SALT					°Mois-	SALT					°Mois-	SALT					°Mois-
	Meth-od 1	Meth-od 2	Meth-od 3	Meth-od 4	Meth-od 5	ture	Meth-od 1	Meth-od 2	Meth-od 3	Meth-od 4	Meth-od 5	ture	Meth-od 1	Meth-od 2	Meth-od 3	Meth-od 4	Meth-od 5	ture
H. R. McMillin, Bur. Animal Industry, Washington, D.C.	2.27 / 2.24	2.27 / 2.30	2.28 / 2.29	2.20 / 2.18		62.20 / 62.10 / 62.10 / 62.10	4.13 / 4.14	4.08 / 4.09	4.09 / 4.09	4.06 / 4.04		63.65 / 63.83 / 63.65 / 63.70	4.13 / 4.13	4.14 / 4.12	4.14 / 4.14	4.03 / 4.04	3.88 / 3.90	47.83 / 47.80 / 47.85 / 47.55
F. B. Hilty, Bur. Animal Industry, Chicago, Ill.	2.27 / 2.24	2.18 / 2.24	2.18 / 2.21	2.18 / 2.21	2.21 / 2.21	61.54	4.05 / 4.09	3.92 / 3.94	3.77 / 3.77	3.92 / 3.92	3.99 / 3.96	64.00	4.24 / 3.99	3.91 / 3.92	3.93 / 3.97	4.07 / 4.10	4.12 / 4.12	47.43
R. H. Philbeck, Bur. Animal Industry, Chicago, Ill.	2.36 / 2.37	2.16 / 2.19	2.21 / 2.22	2.09 / 2.13	2.22 / 2.26	62.15	4.04 / 4.10	4.05 / 3.97	3.97 / 4.02	3.91 / 3.94	4.00 / 4.00	63.70	4.12 / 4.14	4.05 / 4.03	4.09 / 4.07	4.07 / 4.04	4.15 / 4.14	48.50
R. S. Burnett, Cudahy Packing Co., Omaha, Nebr.	2.27 / 2.28	2.11 / 2.06	2.25 / 2.24	2.20 / 2.13		61.90 / 62.00	4.13 / 4.17	3.70 / 3.66	3.82 / 3.84	3.91 / 3.97		63.10 / 63.10	3.82 / 3.84	3.93 / 3.96	3.94 / 3.92	3.82 / 3.91		48.90 / 48.70
W. C. McVey, Bur. Animal Industry, Kansas City, Kansas	2.25 / 2.27	2.26 / 2.25	2.30 / 2.26	2.23 / 2.24	2.25 / 2.26	62.06	4.15 / 4.18	4.08 / 4.03 / 4.10	4.04 / 4.06	3.78 / 3.99 / 3.96	4.06 / 3.97 / 4.12	63.27	4.19 / 4.19	4.14 / 4.14	4.14 / 4.20	3.92 / 3.88 / 3.91 / 3.87	3.84 / 3.90 / 3.90 / 3.88	47.97
H. C. Kershner, Bur. Animal Industry, Kansas City, Kansas	2.24 / 2.25	2.32 / 2.32	2.32 / 2.30	2.28 / 2.26	2.24 / 2.24	61.97	4.09 / 4.08	4.14 / 4.10	4.12 / 4.09	3.64 / 3.78	4.07 / 4.03	63.16	4.17 / 4.08	4.17 / 4.22	4.23 / 4.15	3.89 / 3.85	3.90 / 3.92	48.00
R. C. Newton, Swift & Company, Chicago, Ill.	2.29 / 2.29 / 2.32	2.27 / 2.29 / 2.31	2.28 / 2.26 / 2.27	2.22 / 2.22 / 2.20	2.23 / 2.23 / 2.23	62.00	4.12 / 4.10 / 4.08	4.12 / 4.12 / 4.12	4.15 / 4.12 / 4.12	4.17 / 4.17 / 4.16	4.19 / 4.19 / 4.22	63.60	4.21 / 4.21 / 4.17	4.21 / 4.21 / 4.17	4.17 / 4.17 / 4.21	4.10 / 4.11 / 4.10	4.10 / 4.10 / 4.10	48.94
V. R. Rupp, Int. American Meat Packers, Chicago	2.25 / 2.23	2.35 / 2.32	2.27 / 2.26	2.16 / 2.16	2.32 / 2.28	62.13	4.13 / 4.13	4.14 / 4.16	4.09 / 4.10	3.95 / 3.99	4.08 / 4.07	63.65	4.26 / 4.22	4.22 / 4.20	4.15 / 4.15	4.08 / 4.07	4.25 / 4.23	48.12

David Edelman, Bur. Animal Industry, New York, N.Y.	2.30	2.33	2.27	2.24	2.25	61.88	3.99	4.13	4.06	3.92	4.06	62.95	3.93	3.92	4.13	4.07	4.23	47.84
	2.27	2.29	2.26	2.27	2.28		4.06	4.15	4.07	3.98	4.12		3.98	3.94	4.13	4.01	4.27	
I. M. Myers, Bur. Animal Industry, Omaha, Nebr.	2.23	2.29	2.18	2.23	2.20	62.20	4.04	4.05	4.14	3.99	4.11	63.65	4.07	4.16	4.07	4.05	3.86	49.14
	2.23	2.30	2.24	2.20	2.23		3.99	4.16	4.14	3.97	4.11		4.03	4.14	4.13	3.98	3.96	
	2.23	2.32	2.24	2.21	2.14		4.16	4.09	4.19	4.06	4.04		4.12	4.06	4.16	3.98	4.05	
Wm. C. Owens, Bur. Animal Industry, San Francisco, Calif.	2.27	2.17	2.40	2.39		61.90	4.12	4.13	4.24	4.44		64.10	4.01	4.27	4.30	4.16		48.70
	2.24	2.40	2.38	2.45			4.01	4.08	4.08	4.38			4.13	4.30	4.10	4.09		
Frederick Fenger, Armour & Co., Chicago	2.22	2.25	2.24	2.20	2.23	62.08	4.04	4.07	4.10	4.00	4.06	63.40	4.17	4.19	4.19	4.00	4.13	48.50
W. N. Currier, Campbell Soup Co., Camden, N.J.	2.24	2.28	2.32	2.25		61.00	4.07	4.21	4.21	3.95		62.40	4.10	4.17	4.07	4.16		46.40
	2.22	2.29	2.32	2.24		61.00	4.10	4.22	4.16	3.98		62.10	4.10	4.17	4.03	4.11		46.30
															4.11			
															4.12			
G. L. McGinty, Bur. Animal Industry, St. Louis, Mo.	2.16	2.29	2.26	2.16		61.88	4.07	4.04	4.03	4.09		62.74	4.10	4.15	4.11	4.05		47.43
	2.16	2.29	2.28	2.24		62.09	4.05	4.17	4.03	4.09		62.58	4.13	4.08	4.08	4.07		47.55
W. B. Fromer, Bur. Animal Industry, St. Louis, Mo.	2.18	2.23	2.24	2.18	2.25		4.09	4.09	4.05	4.06	4.09		4.09	4.10	4.09	4.12	3.98	
	2.20	2.29	2.18	2.22	2.23		4.09	4.07	3.95	4.09			4.15	4.05	4.01	4.06	4.01	
C. H. Swanger, Bur. Animal Industry	2.18	2.18	2.17	2.07			4.09	4.07	4.02	3.93			4.11	4.06	4.10	3.93		
	2.25	2.19	2.17	2.07			4.07	4.04	3.97	3.91			4.08	4.09	4.08	3.98		
Maximum	2.37	2.40	2.40	2.45	2.32	62.20	4.18	4.22	4.24	4.44	4.22	64.10	4.26	4.30	4.30	4.16	4.27	48.94
Minimum	2.16	2.11	2.13	2.07	2.14	61.00	3.99	3.66	3.77	3.64	3.96	62.10	3.82	3.91	3.92	3.82	3.84	46.30
Average	2.250	2.260	2.254	2.200	2.238	61.91	4.089	4.069	4.051	4.003	4.081	63.17	4.109	4.077	4.108	4.019	4.033	48.15

* Method 5 in each case was that regularly used by the analyst reporting and accordingly represents a different method in each individual case.

addition and continuing until the solution is colorless or nearly so. Add 25 cc. of distilled water and boil for approximately 5 minutes to eliminate lower oxides of nitrogen. Cool, and dilute to approximately 150 cc. Add 25 cc. of ethyl ether to dissolve the separated fat, and shake. Determine the excess of silver nitrate by titration with standard ammonium or potassium thiocyanate solution, according to the A.O.A.C. method cited in Method 1, omitting the removal of silver chloride by filtration. In the presence of the quantity of ether specified, the well coagulated precipitate of silver chloride does not impair the accuracy of the titration.

Method 3.—Follow Method 2 except to digest the sample with nitric acid by letting it stand overnight on the steam bath.

Method 4.—Weigh out approximately 5 grams of the well comminuted and thoroughly mixed sample in a small beaker, mix to a thin paste with hot distilled water (80°C.), and transfer to a 500 cc. Erlenmeyer flask, washing out the beaker with successive portions of hot water and adding sufficient hot water to bring the volume in the Erlenmeyer to approximately 300–350 cc. Add 25 cc. of concentrated nitric acid and allow to stand on the steam bath 3–4 hours, shaking occasionally. Heat to boiling and add 5 per cent potassium permanganate solution in successive small portions, boiling after each addition until the color of the permanganate has disappeared, and continue until the solution is colorless. Cool, transfer to a 500 cc. volumetric flask, make up to the mark and mix. Determine chlorine in a 100 cc. aliquot by precipitation with silver nitrate and titrate with ammonium or potassium thiocyanate according to the A.O.A.C. method cited under Method 1.

Each collaborator was also requested to determine salt by the method customarily followed in his laboratory. Response was perfect, all collaborators reporting results which bore evidence of careful work and close attention to instructions.

Results show close agreement between Method 1, in which organic matter was destroyed by ignition after addition of sodium carbonate and drying, and Nos. 2 and 3, in which the organic matter was destroyed by wet oxidation with nitric acid and potassium permanganate in the presence of an excess of silver nitrate. It is evident that all three methods yield sufficiently accurate results to justify adoption. In Method 4, the heating with nitric acid apparently involves a slight loss of chlorine.

As the first three methods gave results of equivalent accuracy, the collaborators were requested to express a preference. Responses showed difference of opinion on methods 1 and 2. The recommendation, therefore, is not entirely in accordance with the opinion of all the collaborators.

Because ignition is more commonly employed for the destruction of organic matter than wet oxidation methods, and may possibly be more effective (although the results presented herewith do not indicate that such is the case), it is recommended[1] that Method 1 be adopted as a tentative method with a view to adoption later as an official method.

No report on the separation of meat proteins was given by the associate referee.

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 65, 75 (1933).

REPORT ON GELATIN

By R. M. MEHURIN (Bureau of Animal Industry,
Washington, D. C.), *Referee*

The tentative method for copper in foods tested collaboratively last year with a view to its substitution for a portion of the present tentative method for copper in gelatin was again tested this year in connection with the work on copper and zinc in foods. A full report has been given under the latter heading. Since the limit of accuracy of the tentative method for copper in foods has now been approximately established, attention should be directed to the selection of the most suitable method for the preparation of the sample of gelatin. Three methods are now being used, the ashing, the wet digestion, and the tentative method. The last method involves the precipitation of copper sulfide by passing H_2S through the alkaline solution of the hydrolyzed sample and using ammonium magnesium phosphate as an aid to filtration. Reports of former referees indicated that this method of preparation of the sample is unsatisfactory. In the referee's opinion the most desirable means of destroying the organic matter contained in gelatin is by ashing, without preliminary treatment, in a muffle furnace at a temperature of approximately 500° C. Contamination from the muffle furnace, however, and avoidance of high temperatures during ignition must be guarded against in the determination of the smaller amounts of copper if accuracy is to be attained. The wet digestion method is open to objection only in point of time and attention required for its completion. Experiments are being conducted with a view to shortening this procedure.

The present tentative method for arsenic in gelatin, which involves its precipitation as magnesium ammonium arsenate from its solution in the hydrolyzed sample, does not appear to give dependable results. In his own work, the referee prefers to eliminate this portion of the method and instead to transfer the hydrolyzed sample, after treatment with the customary reagents, directly to the Gutzeit apparatus.

It is recommended[1] that methods for preparation of the sample be studied collaboratively. It is also recommended that the tentative method for arsenic be studied collaboratively.

REPORT ON SPICES AND OTHER CONDIMENTS

By HENRY A. LEPPER (U. S. Food and Drug Administration,
Washington, D. C.), *Referee*

As recommended last year a study of methods for the determination of volatile oils in spices was made by the associate referee, who submits a

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 65 (1933).

report of collaborative work on sage, nutmeg and pimenta. The Clevenger method was studied. It is based on the principle of collecting the volatile oil obtained by steam distillation in a specially designed apparatus. The results, including those of determinations of the physical and chemical constants of the separated oils, led to the conclusion that further work should be undertaken. This recommendation is concurred in by the referee.

Of the recommended studies of methods for mayonnaise, those for solids, oil and acid and for determining the egg content were studied. In this work the referee was assisted by Frank Vorhes, of the Food and Drug Administration, and the results are the subject of the joint supplementary report which follows this report. Recommendations for changes in the methods and for further work are given.

No opportunity was found to study methods for the determination of starch and sugars in prepared mustard and the recommendation of last year on this subject is repeated.

REPORT ON ANALYSIS AND INTERPRETATION OF MAYONNAISE

By HENRY A. LEPPER[1] and FRANK A. VORHES, JR. (U. S. Food and Drug Administration, Washington, D. C.)

At the last meeting of the Association it was recommended "that methods for the determination of solids, oil, sugars and acids, and for determining the egg content of salad dressings be studied."[2] With the exception of methods for sugars, this work has been undertaken.

DEFINITION OF MAYONNAISE

The revision of the Federal standard for mayonnaise[3] made last year altered the regulatory definition of this article. The article must now contain at least 50 per cent vegetable oil, and enough egg to form a stable, semi-solid emulsion. It may contain salt or other seasoning, sugar or dextrose, and the remainder must be undiluted lemon juice and/or vinegar of at least 4 per cent acetic acid strength.[4] The maximum ratio of egg white to egg yolk is limited to that ratio obtaining in whole eggs.

Some types of deviation from the standard which may occur are: (1) Deficiency in vegetable oil, (2) water in excess of that normal to the egg and acid constituents present, (3) excess of egg white, and (4) deficiency of egg to properly form and stabilize the emulsion.

SELECTION OF METHODS

In order to detect the first type the amount of egg yolk must be estimated so that the total fat, as found, may be corrected for the egg fat. It is apparent that the detection of the other three types requires the estimation of egg white as well as yolk.

The Association has, at the present time, no method for the estimation of egg white. The accuracy of the present tentative method for the estimation of egg yolk from lecithin phosphoric acid[1] may be seriously questioned in the light of recent work by Mitchell,[2] Bailey[3] and Perlman.[4] Mitchell reports an enormous loss of lecithin P_2O_5 on bacterial spoilage of mixed eggs. Bailey reports losses of as much as 30 per cent of the lipoid P_2O_5 in mayonnaise stored only 6 weeks. Perlman presents a theoretical mechanism for the loss of lipoid P_2O_5 which involves the production of choline, and supports the theory by finding free choline in suspected samples. His results show that this loss of lipoid P_2O_5 in eggs may be accelerated by shaking. He finds that the loss of lipoid P_2O_5 in mayonnaise is progressive at room temperature and is apparently accompanied by an increase in total lipoids. An additional reason for lack of complete confidence in the lipoid P_2O_5 as an index of egg yolk is the fact that no really extensive authentic data on the lipoid P_2O_5 content of egg yolk, as determined by any one method, were found in the literature.

Recently, however, Mitchell[5] has published an extended study of the composition of eggs, which gives data on solids, fat, total P_2O_5 and total nitrogen as well as other constants on both yolk and white. From this data formulas have been calculated[6] for the estimation of yolk or white and the interpretive error of these formulas established by statistical treatment.

It seemed probable that certain of these formulas might be used for the estimation of the egg components of mayonnaise. To do so it was necessary to use methods for mayonnaise which would give results comparable to those used in obtaining the authentic data on eggs. Accordingly the methods for solids, fat, nitrogen and P_2O_5 were modified only to that extent which would render them applicable to mayonnaise and related products.

METHODS

Total Solids: Use a 2 gram sample and proceed as directed in *2*, p. 244, *Methods of Analysis*, A.O.A.C., 1930.

Total Fat: Use a 2 gram sample and proceed as directed under "Fat (acid hydrolysis)" *This Journal*, **15**, 313 (1932).

Total Acidity: Weigh about 15 grams of sample into a 500 cc. Erlenmeyer flask, dilute to about 200 cc., and shake until all lumps of dressing are thoroughly broken up. Titrate with 0.1 N sodium hydroxide, using neutral phenolphthalein, and cal-

[1] *Methods of Analysis, A.O.A.C.*, 1930, 356, 33.
[2] *This Journal*, 15, 282 (1932).
[3] Connecticut Agr. Exp. Sta. Bull. 341 (1932).
[4] *This Journal*, 15, 467 (1932).
[5] *Ibid.*, 311.
[6] *Ibid.*, 16, 113 (1933).

culate as acetic acid. A duplicate sample should be at hand for comparison in order to detect the very first change of color.

Total Nitrogen: Weigh about 15 grams of sample into a 500 cc. Kjeldahl flask and place on the steam bath until the egg is thoroughly cooked and the oil separates readily. Cool, add about 50 cc. of petroleum ether, mix, and pour off the ether solution through a small filter. Repeat the ether treatment twice, rinsing out as much oil as possible. Wash the filter with petroleum ether and add the filter paper to the sample in the flask. Determine nitrogen using 35 cc. of sulfuric acid for digestion, as directed in 5, p. 245, *Methods of Analysis, A.O.A.C.*, 1930.

Total P_2O_5: Using a 10 gram sample proceed as directed in 16, p. 248, *Methods of Analysis, A.O.A.C.*, 1930, except to use a platinum dish in place of the beaker and to burn off the oil before ashing in the muffle.

Calculation of Composition

When P = % of total P_2O_5 and N = % of total nitrogen, then

% yolk = 75.69 P − 1.802 N;[1]

% white = 60.80 N − 114.59 P;

% total egg = % yolk + % white;

% white in egg component = $\dfrac{\% \text{ white}}{\% \text{ total egg}} \times 100$;

Vegetable oil = total fat − (yolk × 0.3188)*

Vinegar (4% acid strength) = total acidity as acetic × 25;

Minor constituents (sugar, salt, spices, stabilizers) = total solids* − (yolk × 0.5047) − (white × 0.1221)* − vegetable oil; and

Added water = 100% − total egg − vegetable oil − vinegar − minor constituents.

* The constants given in these formulas are the average fat of yolk, average solids of yolk, and average solids of white given by Mitchell (*loc. cit.*) for commercially fresh shell eggs.

EFFECT OF MODIFICATIONS

The use of aluminum dishes for determination of solids on an acid product such as mayonnaise might be questioned. Table 1 gives a comparison of the results obtained by the use of a covered aluminum and an uncovered platinum dish of about the same size. The cover is not regarded as essential as the solids present a smooth, glazed surface, as opposed to the powdery character of most materials which require a covered dish, and do not appear to be hygroscopic. The aluminum dish appears to be as satisfactory as the platinum.

The difficulty of digesting oil for nitrogen determinations and the necessity of using a large sample in order to get a reasonable titer led to the only modification of the method for nitrogen. The question arose as to whether the modification would introduce a loss of egg nitrogen, since it was conceivable that some lipoid egg nitrogen might be soluble in petroleum ether after thorough cooking of the egg. The amount of nitrogen lost by the modification could be ascertained in two ways: (1) The difference between the amount of nitrogen recovered and the theoretical amount of egg nitrogen calculated to be in the sample; and (2) the difference between the amount of nitrogen determined on the ether extract and the amount of oil nitrogen calculated to be in the sample.

[1] *This Journal*, 16, 119 (1933).

TABLE 1

Solids on mayonnaise

SAMPLE	ALUMINUM DISH	PLATINUM DISH	THEORETICAL VALUE[*]
	per cent	*per cent*	*per cent*
1	84.28	84.27	84.08
	84.26		
2	72.54	72.48	72.44
	72.43		
3	73.08	72.93	73.39
	73.13		
4	77.68	77.22	77.57
	77.37		
5	78.41	78.32	78.11
	78.41		
6	60.67	60.69	60.81
	60.67		

[*] Calculated from composition of sample and analysis of materials.

Owing to the small quantities of nitrogen to be handled, experiments were made both ways in duplicate, and the four results were averaged. Some of the oil will not be removed, but non-volatile matter determined on a few ether extractions, as made in the modification, indicated that roughly 90 per cent of it was removed. Table 2 gives results obtained and comparative calculations. It is evident that the egg nitrogen lost by the ether extraction is negligible and is probably more than compensated for by the nitrogen derived from spices.

The method for P_2O_5 in eggs was modified only to the extent that a platinum dish was required in order that the oil might be burned off before the sample was placed in the muffle. Otherwise the oil volatilizes in the muffle and is liable to explode.

The present tentative method for total acidity in salad dressings was altered to allow the use of a larger sample for the sake of larger titer and correspondingly greater accuracy. Trials indicated that the end point was easily recognized by comparison with a duplicate sample, even when the larger sample was diluted to only 200 cc. instead of to 400 cc.

The method for total fat in eggs was adopted without change.

PREPARATION OF COLLABORATIVE SAMPLES

It was desirable to test these methods collaboratively on samples of varying composition. Accordingly six samples were prepared to represent several deviations from the mayonnaise standard, as well as normal types.

TABLE 2

Effect of modification on nitrogen method.§

SAMPLE	TOTAL N		EGG NITROGEN		ETHER EXTRACT N		EGG N IN ETHER EXTRACT	AV. EGG N LOST	% EGG N LOST
	DETERMINED (1)	CALCULATED* (2)	CALCULATED (3)	NOT RECOVERED (3)-(1) (4)	DETERMINED (5)	CALCULATED† (6)	(5)-(6) (7)	AV. (4) & (7) (8)	(8)×100 / (9) (9)
1	0.1305	0.1296	0.1294	-0.0011	0.0048	0.0023	0.0025	0.0013	1.0
	0.1281			0.0013	0.0048		0.0025		
2	0.5243	0.5365	0.5364	0.0121	0.0154	0.0009	0.0145	0.0136	2.5
	0.5248			0.0116	0.0170		0.0161		
3	0.2878	0.2907	0.2906	0.0028	0.0041	0.0009	0.0032	0.0062	2.1
	0.2741			0.0165	0.0031		0.0022		
4	0.2723	0.2772‡	0.2613	-0.0111	0.0024	0.0021	0.0003		
	0.2736			-0.0123	0.0021		0.0000		

* Egg N +0.1 Oil N.
† 0.9 Oil N.
‡ Including spice nitrogen.
§ Results on all but last column are given as percentage of sample. Samples 1, 2 and 3 contained no spices. Sample 4 contained 0.256 per cent dry mustard, probably an average amount. The fourth decimal to which the results are carried is not intended to show great accuracy, but rather to assist in indicating the trend.

For the different samples the following materials were used and the analyses indicated were made.

MATERIAL	DESCRIPTION		SOLIDS	NITROGEN	P₂O₅	FAT
			per cent	*per cent*	*per cent*	*per cent*
Eggs	1 day old, perfect in appearance	{Yolks	51.38,51.42	2.688,2.694	1.383	32.29,32.28
		{Whites	12.70,12.69	1.792,1 798	0 04 assumed for calc	
	Less than 3 days old, high grade commercially fresh	{Yolks	50.51,50 63	2.526,2.522	1.336,1 337	32 48,32.18
		{Whites	12.32,12.27	1.736,1.728	0.04 assumed for calc.	
	Age unknown—probably good grade of storage egg	{Yolks	49.26,49.28	2.555,2.574,2.554	1.304,1.300	31 00,31.04
		{Whites	12.53,12 50	1.790,1.772	0.04 assumed for calc.	
Oil	Corn oil—retail package			0.0031	0.0022,0.0015	
	Cottonseed oil—retail package			0.0015	0.0005,0.0007	
Salt	C.P. NaCl.					
Spice	Dry mustard—retail package			5.40,5 40	2.30,2.29	
Acid	10% acetic acid was used instead of vinegar for reasons given					

The method of preparation is as follows:

The bowl and beater of a small sized Hobart mixer was weighed to 0.01 oz. The egg yolk, salt, and spice (if any) were weighed by difference to 0.05 gram, and placed in the bowl. Bowl and beater were attached to the machine and the mixture was stirred slowly with just enough water to produce a smooth batter. A separatory funnel was filled with oil and weighed to 0.5 gram. The funnel was supported by a ring stand over the side of the bowl and the oil added in a small stream while the batch was rapidly mixed. From time to time a small quantity of water was added to prevent the batch becoming too stiff. When the oil was completely added, the separatory funnel was weighed and the quantity of oil was obtained by difference. Then the egg white was weighed in by difference to 0.05 gram, and somewhat more than the required quantity of water added and mixed in slowly. The required amount of acid was then added from a pipet and mixed in. The sides were scraped down with the beater and care was taken to eliminate any non-uniformities in the batch that were visible. The bowl with batch and beater were then weighed to 0.5 gram, and the tare on the bowl and beater was subtracted to obtain the weight of the batch. The batch was immediately transferred to three one-pint Mason jars. The combined weight of all materials except water was subtracted from the weight of the batch to obtain the water actually present. The loss of water due to evaporation while mixing amounted to 20 to 40 grams on a 1500 gram batch. If vinegar instead of the 10 per cent acetic acid had been used it would have been necessary to add it from time to time during the mixing, with accompanying loss of acidity by evaporation. The method of preparation used probably allows only a negligible loss of acidity. The amount of nitrogen or phosphorus contributed by cider vinegar in commercial mayonnaise is quite negligible. For these reasons it is considered that the batches prepared are comparable even to those which may contain fruit vinegars. Distilled vinegar containing relatively little of these constituents is the customary commercial acid component. Batches 1, 2 and 3 contained no spice. Batches 4, 5 and 6 contained 0.256, 0.222 and 0.304 per cent of dry mustard, respectively. Sample 6 also contained 4.05 per cent of starch, which was added as a paste at the start of the preparation. The proportion of other ingredients is given in the "Theory" columns of Table 3, as actually prepared (not calculated from the theoretical analysis).

TABLE 3

Collaborative results

COLLABORATOR	SAMPLE 1 EGGS—AGE UNKNOWN—CORN OIL			SAMPLE 2 EGGS—AGE UNKNOWN—COTTONSEED OIL			SAMPLE 3 EGGS SAID TO BE 3 DAYS OLD—COTTONSEED OIL—NO SPICE			
	B	A	THEORY	D	A	THEORY	D	A	C	THEORY
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Solids	84.34 84.38 84.24	84.28 84.26	84.08	72.92 72.90 72.97	72.54 72.43	72.44	73.44 73.53 73.50	74.09 74.02	73.08 73.13	73.39
Fat	83.31 83.11 83.34	83.20 83.45	82.65	68.80 68.58 68.77	68.04 67.87	67.51	70.63 70.90 71.14	71.39 71.35	71.25 71.11	70.31
Acidity (as HAc)	0.58 0.58 0.58	0.584 0.586	0.593	0.816 0.816 0.828	0.770 0.782	0.765	0.398 0.400	0.398 0.389	0.392 0.395	0.412
Total P$_2$O$_5$	0.063 0.062 0.064	0.058 0.059	0.057*	0.247 0.247 0.245	0.246 0.240	0.245*	0.065 0.067	0.061 0.063	0.067 0.065	*0.064
Total nitrogen	0.129 0.130 0.128	0.130 0.128	0.129†	0.502 0.508	0.524 0.525	0.536†	0.284 0.286	0.286 0.289	0.288 0.274	†0.290
CALCULATIONS (on averages)										
Yolk	4.53	4.20	4.22	17.63	17.48	18.68	4.48	4.17	4.47	4.48
White	0.64	1.14	1.20	2.69	4.03	3.26	9.77	10.41	9.56	10.25
Total egg	5.17	5.34	5.42	20.32	21.51	21.94	14.25	14.58	14.03	14.72
White in egg component	12.4	21.3	22.1	13.2	18.7	14.9	68.6	71.4	68.1	69.6
Vegetable oil	81.81	81.97	81.34	63.06	62.39	61.71	69.46	70.04	69.75	68.86
Vinegar (4% HAc)	14.5	14.6	14.8	20.5	19.4	19.1	10.0	9.85	9.85	10.3
Minor constituent	0.15	0.04	0.51	0.64	0.78	1.11	0.54	0.64	(−.15)	1.00
Added water	(−1.6)	(−2.0)	(−2.1)	(−4.5)	(−4.1)	(−3.9)	5.8	4.9	6.5	5.1

* Total P$_2$O$_5$ including P$_2$O$_5$ of oil.
† Theoretical egg nitrogen.

COLLABORATOR	SAMPLE 4 — EGGS SAID TO BE 3 DAYS OLD—CORN OIL				SAMPLE 5 — 1 DAY OLD PULLET EGGS—COTTONSEED OIL				SAMPLE 6 — 1 DAY OLD PULLET EGGS—50–50 COTTONSEED AND CORN OIL			
	B	C	A	THEORY	F	B	A	THEORY	F	B	A	THEORY
	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent	per cent
Solids	77.54	78.33	77.68	77.57	79.09	78.21	78.41	78.11	60.57	60.50	60.67	60.81
	77.64	78.37	77.37		78.89		78.41		60.56		60.67	
	77.56											
Fat	75.39	76.47	75.89	74.93*	77.79	76.92	76.82	76.18*	55.39	55.11	54.74	54.16*
	75.43	76.74	75.82		77.93		77.12		55.28		54.48	
	75.42											
Acidity (as HAc)	0.43	0.413	0.416	0.423	0.85	0.86	0.865	0.848	0.43	0.45	0.435	0.418
	0.43	0.416	0.413				0.860				0.435	
	0.43											
Total P_2O_5	0.076	0.073	0.072	0.074†	0.092	0.087	0.083	0.087†	0.112	0.104	0.105	0.104†
	0.077	0.073	0.071		0.093		0.086		0.112		0.105	
	0.076											
Total nitrogen	0.266	0.268	0.272	0.275‡	0.187	0.175	0.188	0.187‡	0.264	0.241	0.255	0.285‡
	0.266	0.271	0.274		0.185		0.190		0.257		0.257	
	0.271											
CALCULATIONS (on averages)												
Yolk	5.29	5.04	4.90	4.78	6.70	6.27	6.08	5.83	8.01	7.44	7.49	6.88
White	7.55	8.05	8.44	8.12	.65	.67	1.77	1.06	3.04	2.74	3.53	2.96
Total egg	12.84	13.09	13.34	12.90	7.35	6.84	7.85	6.89	11.05	10.18	11.02	9.84
White in egg component	58.8	61.5	63.3	62.9	8.8	9.8	22.5	15.4	27.5	26.9	32.0	30.1
Vegetable oil	73.72	75.00	74.30	73.38	75.72	74.92	75.03	74.30	52.79	52.74	52.22	51.94
Vinegar (4% HAc)	10.8	10.4	10.4	10.6	21.25	21.5	21.6	21.2	10.75	11.25	10.9	10.45
Minor constituent	.27	(−.18)	(−.27)	.77	(−.19)	.04	.09	.74	3.36	3.67	4.24	4.96
Added water	2.4	1.7	2.2	2.4	(−4.1)	(−3.3)	(−4.6)	(−3.1)	22.2	22.2	21.6	22.8

* From egg and vegetable oil—neglecting spices.
† Total P_2O_5 from oil, egg and spices.
‡ Theoretical egg nitrogen plus spice nitrogen.

DISCUSSION OF RESULTS

It should be mentioned that the deviation of the results obtained from the theoretical results represents not only the error of analysis and interpretation, but also the error of synthesis of the samples, the error or sampling, and the error of analysis of materials. Therefore it is considered that as a whole the deviations from the theory represent greater errors than would usually occur in practice.

The method for fat appears to give consistently high results, which are shown in a high calculation of vegetable oil and a low calculation of minor constituents. These errors of interpretation are compensating, however, in the calculation of added water. The results obtained are more consistent and closer to the theoretical value than any reported in previous collaborative work.[1]

In view of the small percentages of nitrogen and phosphorus to be determined, it is felt that the results obtained by these methods are quite satisfactory. Here again, on interpretation, errors of analysis, as well as the effect of spices tend to be compensating, especially in calculation of total egg. The methods for total solids and acidity are evidently quite satisfactory. Among the calculations the most striking is probably that of added water. The results given as negative indicate a strength of acid greater than 4 per cent.

Appreciation is acknowledged to the following collaborators of the U. S. Food and Drug Administration, whose results are identified as follows: B—Samuel Alfend, St. Louis; C—E. H. Berry, Chicago; D—A. K. Klein, San Francisco; E—C. D. Schiffman, New York; and F—F. L. Elliot, Baltimore. Results listed under Collaborator A were obtained by the writers.

RECOMMENDATIONS[2]

It is recommended—

(1) That the present tentative methods for the determination of total solids, total acidity, lecithin P_2O_5 and egg solids be deleted.

(2) That the methods presented for the determination of total solids, total acidity, total nitrogen and total P_2O_5 be adopted as official (first action).

(3) That the method presented for the determination of total fat be adopted as tentative.

(4) That the methods of calculation of composition given be adopted as tentative.

(5) That study on methods for salad dressings be continued.

[1] *This Journal,* 7, 189 (1923); 8, 172 (1924); 8, 700 (1925).
[2] For report of Subcommittee C and action of the Association, see *This Journal,* 16, 66 (1933).

REPORT ON VOLATILE OIL IN SPICES

By J. F. CLEVENGER (U. S. Food and Drug Administration,
New York, N. Y.), *Associate Referee*

A method for the determination of volatile oil in various plant products outlined by Clevenger[1] provides for the direct reading of the amount of volatile oil obtained from the plant material and a determination of certain physical and chemical characteristics of the oil thus obtained. In this method it was recognized that the different plant materials require a variation in technic, depending upon the character of the material from which the volatile oil is obtained and the specific gravity of the volatile oil thus obtained.

COLLABORATIVE WORK

In order to determine the practicability of this method, an attempt was made to mix uniformly whole sage, put in two pound, double, paper bags, and ground sage, nutmeg, and pimenta put in suitably sealed glass jars. The following collaborators assisted:

O. C. Kenworthy and J. Fitelson, New York Station.
S. Alfend, St. Louis Station.
L. H. McRoberts, San Francisco Station.
J. H. Cannon, the Chicago Station.
L. Hart, Food Control Laboratory.

Approximate conditions found suitable for distillation of volatile oils in the following products:

PRODUCT	AMOUNT USED	FLASK (SIZE)	H₂O ADDED	TIME DISTILLED
	gram	*cc.*	*cc.*	*hours*
Sage (whole)	100	2000	500	4
Sage (ground)	150	2000	600	4
Nutmeg (ground)	25	500	200	4
Pimenta (ground)	100	1000	400	5

The collaborators were requested to observe the following cautions and suggestions:

1. Mix uniformly the plant product and water in the flask before beginning the distillation of the volatile oil.

2. Use type of volatile oil trap required for the oil contained in the plant product.

3. Take care that the distillation is conducted at a rate sufficiently slow to prevent the escape of vapors around the condenser, thus insuring against loss of volatile oil.

4. In case of unsatisfactory separation of the volatile oil in the trap the contents of the trap (oil and water mixture) may be drawn off in a small separatory funnel and allowed to separate, the water being returned to the trap and the volatile oil transferred to a small graduate cylinder. This operation may be repeated as often as conditions require. Finally the amount of the volatile oil obtained may be read directly in the cylinder, and the percentage of volatile oil present in the plant product calculated.

[1] *J. Am. Pharm. Assoc.*, **17**, 345 (1928).

5. Obtain the following constants for oils of sage, nutmeg and pimenta: specific gravity at 25°/25°C., optical rotation at 25°C., refractive index at 20°C., eugenol, p. 427, acid, and ester number (see U.S.P.X.).

6. Use *short necked* flasks for distillation.

7. Reduce condensation by a fold of asbestos board (1/16 in. thick is suitable) around the *neck of flask and connecting tube of trap.*

Results of assay.

	CLEVENGER	KENWORTHY	FITZLSON	ALFEND	MCROBERTS	CANNON	HART
			SAGE (UNGROUND)				
Yield	1.9%	2.0	2.1	2.1	1.9	1.2*	1.77
Sp. Gr.	0.926	0.924	0.921	0.916	0.921	0.919	0.921
Op. Rot.	+1.4	+1.2	+1.8	+0.7	—	+16.0	+1.86°
Ref. Ind.	1.465	1.468	1.464	1.463	1.465	1.462	1.469
(Acid Number	2.4	2.6	1.36	1.0	1.3	0.94	—
(Ester Number	19.5	24.7	10.25	9.0	11.7	13.6	—
			SAGE (GROUND)				
Yield	1.5	1.3	1.5	1.8	1.5	1.1*	1.34
Sp. Gr.	0.930	0.920	0.926	0.915	0.926	0.919	0.921
Op. Rot.	+2.4	+2.2	+3.1	+3.0	—	+16.0	+5.19
Ref. Ind.	1.465	1.466	1.463	1.465	1.464	1.462	1.469
(Acid Number	2.3	1.0	1.2	1.0	1.4	0.94	—
(Ester Number	13.1	18.3	16.8	9.0	12.4	13.6	—
			NUTMEG (GROUND)				
Yield	8.1†	8.8	9.8	9.6	9.1	6.7	8.1
Sp. Gr.	0.930	0.926	9.920	0.913	0.917	0.898	0.920
Op. Rot.	+10.0	+17.5	+16.5	+16.0	—	+17.9	+14.0
Ref. Ind.	1.485	1.483	1.484	1.485	1.485	1.482	1.487
(Acid Number	11.2	2.4	4.3	3.5	3.7	1.2	—
(Ester Number	13.0	7.7	6.5	4.6	2.9	10.2	—
			PIMENTA (GROUND)				
Yield	4.2	4.3	4.0	4.1	2.75	2.9	2.7
Sp. Gr.	1.039	1.039	1.039	1.039	1.037	1.037	1.037
Op. Rot.	−3.0	−2.6	−1.7	−3.7	—	−1.9	−1.3(?)
Ref. Ind.	1.531	1.533	1.531	1.532	1.530	1.532	1.535
Eugenol‡	84	84	88	88	81	83	82

* Physical and chemical constants were determined for combined oils.
† Analysis made 5 months after the nutmegs were ground and stored in sealed glass jars.
‡ The eugenol content was determined by using a Babcock milk bottle, 2 cc. of volatile oil, and proportionate amounts of the needed reagent.

DISCUSSION

Reasonable agreement in yield of volatile oil was obtained in most instances. The lower yields indicate lack of uniformity in mixing, incomplete distillation, or the loss of some volatile oil in the steam during distillation. In the majority of instances uniformity of mixture is indicated in the yields of oil.

The length of the neck of the flask used for the distillation influences not only the yield but also the character of the volatile oil. Prolonged distillations of materials containing fixed oils heavier than water in addition to the volatile oils will include small quantities of the more volatile fractions of fixed oil. Nutmeg is an illustration. It is therefore recom-

mended that the oil be drawn off in a small separatory funnel after about 4 hours of distillation and at approximately 30 minute intervals thereafter, and that the distillation be discontinued when the fraction of oil obtained is heavier than water.

As the yield is reported in cc. obtained per 100 grams of material taken it is possible that a variation in the calibrations of graduated cylinders used for measuring the oils might account for some variation in this determination. A percentage yield based upon the weight of the volatile oil obtained might be preferable.

As it is necessary, in most instances, to determine first the physical constants and afterwards the chemical characteristics on a few cc. of volatile oil, it is important to use care to reduce to a minimum the loss of oil in the subsequent determinations. Variations in the specific gravities and refractive indices may be due in part to incomplete distillation of the volatile oils, as the last fractions of oils thus obtained have higher specific gravities and higher refractive indices. In general, the fractions of optically active volatile oils vary in their activity, the lighter fractions usually being more active. However, this should not account for the high activity reported by Cannon for volatile oil of sage. No doubt the lack of a sharp end point in the determination of the acid number for volatile oils accounts for most of the variations reported.

As most essential oils contain substances that affect KOH (easily dehydrated alcohols, aldehydes and polymerizable substances) it is essential that the one-hour saponification be strictly followed.

It is recommended[1] that further work be done.

REPORT ON CACAO PRODUCTS

By J. W. SALE (U. S. Food and Drug Administration,
Washington, D. C.), *Referee*

The associate referees on milk proteins (in milk chocolate), on cacao butter, and on sucrose and lactose, made marked progress this year in perfecting methods for the analysis of cacao products.

Milk proteins in milk chocolate: Offutt's new procedure for the determination of milk proteins in milk chocolate has so many advantages over the present tentative method that some consideration was given to recommending that it be made official. However, it was decided that additional data should be obtained. The sample is defatted, which facilitates the action of the solvents and reduces the foaming propensities in the digestion.

Cacao butter: Winkler submitted mixtures of fats of known composition to collaborators, who obtained results which justify the conclusion

that further work on the method for the determination of the "A" and "B" numbers is warranted. The advantages of this method of detecting and determining foreign fat in chocolate products are threefold. The "A" number of cocoa butter is zero, which is not true of many other constants. The "A" number is almost directly proportional to the amount of coconut or palm kernel oil, which is not the case with the Reichert-Meissl and Polenske values, the former being increased and the latter reduced from the calculated amount. The spread between the constants in this method is greater than that for most constants.

Sucrose and lactose in milk chocolate: Abundant collaborative work (9 collaborators) on the new methods for sucrose and lactose, which are very much needed, was conducted. The new polarimetric method for sucrose is only slightly different from the present accurate tentative method. The new method for the determination of lactose by copper reduction gives results which are more uniform and dependable than those obtained by the present tentative polarimetric procedure.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method for the determination of crude fiber in milk chocolates[2] be adopted as official (final action).

(2) That further work be done on the method for the determination of milk proteins in milk chocolate described by the associate referee.

(3) That the method for the quantitative determination of foreign fat by means of the "A" and "B" numbers, described in the 1931 report on cacao butter,[3] be further studied.

(4) That the methods for the determination of sucrose in chocolate and lactose in milk chocolate described in the report for 1931 on sucrose and lactose[4] be adopted as tentative.

(5) That further collaborative work be done on the proposed methods for sucrose and lactose, with a view to making them official.

(6) That the present tentative method for sucrose and lactose in cacao products be dropped.

REPORT ON MILK PROTEINS IN MILK CHOCOLATE

By MARIE L. OFFUTT (U. S. Food and Drug Administration, New York), *Associate Referee*

An attempt was made to obtain a method which would eliminate some of the undesirable features of the present method and give more accurate collaborative results.

The first change which seemed to be of value was the removal of the

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 66 (1933).
[2] *This Journal*, 15, 72 (1932).
[3] *Ibid.*, 548.
[4] *Ibid.*, 556.

fat to permit a better solvent action and to lessen the foaming in the nitrogen digestion. The treatment of the material with solvents in cold and hot condition was also tried as it was noticed in the work on cacao products[1] that both liquor and sweet chocolate contained casein. The amount of cacao material extracted and later precipitated as casein was found to vary according to the interpretation by the analyst of the phrase "few minutes." Varying strengths of sodium oxalate and of time of standing in cold were tried, as well as the sodium tetraborate solution specified in the Lepper-Waterman method.[2] The sodium tetraborate acted as did the hot oxalate in extracting some cacao material which is precipitated by the acid. Table 1 shows the results by the present A.O.A.C. method and by the proposed method on some liquors, sweet chocolates and milk chocolates.

TABLE 1

Milk protein

(Casein × 1.25)

SAMPLE	A.O.A.C	PROPOSED METHOD	CALCULATED
	per cent	*per cent*	*per cent*
Liquor B	2.69	0.03	None
C	1.18	0 05	None
D	2.35	0.16	None
E	0.70	0 03	None
S	2.32	0.15	None
W	1.74	0.03	None
X	0.81	0 11	None
Sweet Chocolate			
1	0.84	0.26	None
2	0.61	0.28	None
3	1.03	0.25	None
Milk Chocolate	2.41	2.86	2.54
1	4.38	4 15	4.26
2	3.43	3.58	3.33
3	3.50	2.68	2.79
4	3.05	2.70	2.66
5	1.64	1.89	1.97

The calculated milk protein figures given in this report were obtained by multiplying the casein figures of the proposed method by 1.25, since the milk protein of some milk powders determined in this way gave close checks to the milk protein (N × 6.38) results determined by nitrogen directly.

Two samples of milk chocolates were sent to collaborators with instructions: to determine the milk proteins by the tentative method (Casein in milk chocolate), where casein×1.25 gives milk proteins, and by the following proposed method.

[1] *This Journal*, **9**, 470 (1926).
[2] *Ind. Eng. Chem.*, **19**, 501 (1927).

TABLE 2
Percentage of milk protein (Casein × 1.25)

COLLABORATOR	SAMPLE X—(CALCULATED 4.26)				SAMPLE Y—(CALCULATED 3.33)			
	A.O.A.C. METHOD		PROPOSED METHOD		A.O.A.C. METHOD		PROPOSED METHOD	
R. U. Bonnar	3.80		4.47		3.25		3.38	
	3.90	Av. 3.85	4.61	Av. 4.54	3.54	Av. 3.40	3.45	Av. 3.41
W. O. Winkler	4.39		4.78		3.59		3.49	
	4.46	Av. 4.43	4.91	Av. 4.85	3.91	Av. 3.75		3.49
D W. McLaren	2.90		4.28		2.72		3.44	
	3.45		4.15	Av. 4.21	2.61	Av. 2.67	3.14	Av. 3.37
	3.41						*3.54	
	2.99	Av. 3.19						
W. T. Mathis	4.19		4.40		3.35		3.48	
	4.19	Av. 4.19	4.40	Av. 4.40	3.27	Av. 3.31	3.35	Av. 3.41
M. L. Offutt	4.38		4.05		3.43		3.58	
	3.73	Av. 4.06	4.10	Av. 4.08	3.08	Av. 3.26	3.35	Av. 3.47
J. Fitelson	4.69		4.46		3.43	3.43	3.20	3.20
	4.43	Av. 4.56	4.69	Av. 4.58				
T. B. Benjamin	3.64		4.35		2.68		3.21	
	3.58	Av. 3.61	4.35	Av. 4.35	2.65	Av. 2.67	3.30	Av. 3.26
*Maximum	4.56		4.85		3.75		3.49	
Minimum	3.19		4.08		2.67		3.20	
Average	3.98		4.42		3.21		3.37	
Variation	1.37		0.77		1.08		0.29	

* Shaking machine 2 hours.

PROPOSED METHOD

Special Reagent.—3% Sodium Oxalate Solution.

Weigh exactly 10 grams of the finely grated chocolate into a suitable 8 ounce centrifuge bottle. Add two 100 cc. portions of ether, centrifugalize and decant the supernatant liquor. Dry the residue in an oven at about 100°C. and powder the residue in the bottle with a flattened glass rod. Add 200 cc. of the reagent and let stand 4 hours, shaking frequently. Centrifugalize and filter through a small folded filter. Discard the first 5–10 cc. of the filtrate and determine nitrogen in 50 cc. of this filtrate. Pipet 100 cc. of the filtrate into a 200 cc. volumetric flask and dilute almost to the mark with water. Precipitate the proteins by the addition of 2 cc. of glacial acetic acid. Make to volume, shake, filter, and determine nitrogen in 100 cc. of the filtrate. The difference between the two nitrogen figures obtained above is the nitrogen of the milk proteins contained in 2.5 grams of the sample. This figure ×4×6.38 gives the total casein contained in the 10 grams taken for the analysis. Casein×1.25 gives total milk protein.

The chemists reporting and to whom acknowledgment is made are:—

W. T. Mathis, Conn. Agr. Expt. Station, New Haven, Conn.
D. W. McLaren, Food and Drug Administration, Buffalo, N. Y.
W. O. Winkler, Food and Drug Administration, Washington, D. C.
R. V. Bonnar, Food and Drug Administration, San Francisco, Calif.
J. Fitelson, Food and Drug Administration, New York, N. Y.
T. B. Benjamin, Food and Drug Administration, Chicago, Ill.

COMMENTS BY COLLABORATORS

McLaren suggests that mixing on a shaking machine for 2 hours gives the same results as does standing for 4 hours. He also found that prolonged standing in the sodium oxalate solution gave low results. W. O. Winkler suggests that the time of centrifugalizing be specified and that possibly some inert material could be added to the sample to carry down the finer particles. The results obtained by the collaborators are given in Table 2.

The results in Table 2 seem to indicate that a more uniform agreement among analysts is obtained by the proposed method, which would be of value in calculating total milk solids in milk chocolate.

It is recommended[1] that further collaborative work be done on the proposed method for the determination of milk proteins in milk chocolates.

REPORT ON CACAO BUTTER

By W. O. WINKLER (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The method for the determination of the "A" and "B" numbers described in the report on cacao butter for 1931[2] was further tested. It was modified in certain particulars and submitted to collaborators, together with three samples. One sample consisted of refined whole coconut

[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 66 (1933).
[2] *This Journal*, 15, 548 (1932).

oil, another of hydrogenated plastic coconut butter, and a third of a mixture of 90 per cent cocoa butter and 10 per cent refined whole coconut oil. Of the five collaborators reporting results, three obtained fairly concordant data, but the data submitted by the other two were unsatisfactory and clearly indicated unfamiliarity with the procedure. The data and reports submitted indicate that the directions for the saponification of the fat should be presented in greater detail and that the description of certain other steps in the method should be clarified. The associate referee believes that the procedure has merit, but he is not including the modified procedure or the data submitted by collaborators in this report because it is obvious that the collaborators should be supplied with more specific directions than were sent to them.

It is recommended[1] that the method for the quantitative determination of foreign fat by means of "A" and "B" values described in the report on cacao butter for 1931 be further studied.

REPORT ON SUCROSE AND LACTOSE IN MILK CHOCOLATE

By J. FITELSON (U. S. Food and Drug Administration, New York, N. Y.), *Associate Referee*

In accordance with the recommendations for 1931, two collaborative samples of authentic milk chocolate were sent out for analysis, the methods used being those described in the previous report.[2] Table 1 shows the results obtained by the collaborators.

Results for sucrose by the proposed method, which differs only slightly from the present tentative method, are quite satisfactory. The maximum variation from the calculated percentage was +0.80 per cent sucrose, with an average variation, disregarding algebraic signs, of 0.28 per cent. Polariscopic results for lactose varied considerably, with a maximum variation of 34 per cent of the calculated lactose present. Results for lactose determined by the copper reduction method yielded a maximum variation of 14 per cent of the accepted figure. The average variation for the proposed method for lactose is 0.15 per cent, whereas that for the polariscopic method is 0.28 per cent lactose. Results for the polariscopic method for lactose agree well with those found in previous collaborative experiments; results by the copper reduction method are in general in better agreement with the true figures. If the two extreme low results are disregarded, the agreement is usually within +0.3 per cent. Since the method tends to yield results about 0.3 per cent high, owing to the reducing substances present in cacao, these low results can be justifiably viewed with suspicion.

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[1] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 66 (1932).
[2] *This Journal*, 15, 556 (1932).

TABLE 1

Collaborative results

COLLABORATOR	SAMPLE N (Sucrose present—48.15%) (Lactose present—3.48%)			SAMPLE T (Sucrose present—46 00%) (Lactose present—4 56%)		
	SUCROSE FOUND	LACTOSE FOUND BY—		SUCROSE FOUND	LACTOSE FOUND BY—	
		POLARI-SCOPE	COPPER REDUCTION		POLARI-SCOPE	COPPER REDUCTION
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
P. A. Mills	47.89	3.54	3 42	46.77	5.56	4.75
	46.59	4.04	3.53	46.35	6.10	4 69
Average	47.74	3.79	3.47	46 56	5 83	4.72
W. T. Mathis	47 93	3.45	3 36	46 28	4 73	4 67
	47.82	3.64	3 49	46.22	4 79	4.71
Average	47.88	3.55	3.43	46.25	4.76	4 69
M. L. Offutt	48.13	3.63	3.83	46 20	4 34	4.55
	48.03	3.59	3.38	46 39	4 42	4.88
Average	48.08	3.61	3 61	46 30	4 38	4 72
L. H. McRoberts	48.16	3 60	3.44	46 50	5 05	4.76
	47.69	4.05	3.60	46 71	4 54	4.57
	48.68	3.75	3.46	46.11	4.90	4 73
						4 87
Average	48.18	3.80	3 50	46.44	4.83	4.73
M. M. Jackson	48.16	3.93	3.01	46 62	5.10	4 13
	48.16	3.93	3.08	46.80	4 87	4 08
Average	48.16	3.93	3 04	46 71	4.99	4.10
W. O. Winkler	48.28	3.75	3.40	46.39	4 85	4 57
	48.22	3.66	3.41	46.01	5.36	4.59
Average	48.25	3.71	3 41	46.20	5 10	4 58
J. Fitelson	48 23	3.51	3.36	46 14	4 90	4 62
D. W. McLaren	47.91	4.48	3.61	45 63	5 88	4 94
	47.44	4.42	3 72	46 00	5 74	4.76
Average	47.68	4.45	3 67	45 82	5.81	4 85
T. B. Benjamin	48.40	4.28	3 53	46 00	5 73	4.49
	48.55	4.09	3.48	45 98	5 46	4 47
Average	48.47	4.19	3 52	45 99	5 59	4 48
Maximum	48.68	4.48	3.83	46 80	6 10	4 94
Minimum	47.44	3 45	3.01	45 63	4 34	4 08
Average	48.07	3.85	3.45	46 28	5 13	4 62
Max. Variation	−0.71	+1.00	−0.47	+0.80	+1 54	−0.48
Av. Variation	0.24	0.23	0.13	0 33	0.32	0 17

Among the pertinent comments received from the collaborators were some suggesting removal of lead before the direct polarization. The use of hot water to effect solution of the sugars met with approval. The presence of lead in the direct polarization does not affect the reading appreciably. For the sake of uniformity, however, the methods were changed in this and in a few other respects. The revised methods follow:

SUCROSE

Transfer 26 grams of the sample, prepared as directed under XIX, 1[1], to an 8 ounce nursing bottle, add about 100 cc. of petroleum ether, shake 5 minutes and centrifugalize. Decant the clear solvent carefully and repeat the treatment with petroleum ether. Place the bottle containing the defatted residue in a warm place until the petroleum ether is expelled. Add 100 cc. of water and shake until most of the chocolate is detached from the sides and bottom of the bottle. Loosen the stopper and carefully immerse the bottle for 15 minutes in a water bath kept at 85°–90°C, shaking occasionally to remove all the chocolate from the sides of the bottle. Re-

[1] *Methods of Analysis, A.O.A.C.*, 1930.

move from the water bath, cool, and add basic lead acetate solution (sp. gr. 1.25) to complete precipitation (5 cc. is usually sufficient). Add water to make a total volume of 110 cc. of added liquid. Mix thoroughly, centrifugalize and decant the supernatant liquid through a small filter. Precipitate the excess of lead with powdered dry potassium oxalate and filter. Dilute sufficient filtrate with an equal volume of water, mix and polarize in a 200 mm. tube at 20°C. Obtain the invert reading at 20°C. as directed under XXXIV, 23 (b) Multiply both readings by 2 to correct for dilution. From the data obtained calculate the percentage of sucrose (S) from the following formulas:

$$S = \frac{(P-I)(110+X)}{143.0 - t/2}, \text{ in which the value of } X \text{ is obtained from}$$

$$X = \frac{0.2244 \ (P-21d)}{1 - 0.00204 \ (P-21d)}, \text{ in which the value of } d \text{ is obtained from}$$

$$d = \frac{P-I}{143.0 - t/2}.$$

LACTOSE

Determine reducing sugars before inversion as directed under XXXIV, **38**, in an aliquot (usually 20 cc.) of the lead-free filtrate obtained in the sucrose determination. Reduced copper as cuprous oxide should be determined by the volumetric thiosulfate method as directed under XXXIV, **41**. Correct for the cuprous oxide due to the sucrose as follows:

Obtain the approximate percentage of lactose from the following formula, using the data obtained in the Sucrose (S) estimation:

$$\text{Approximate lactose} = \frac{P(1.1 + X/100) - S}{0.79}.$$

From the calculated polarimetric sucrose/lactose ratio and the total cuprous oxide obtained as above, determine the amount of cuprous oxide to be subtracted from the total cuprous oxide found, using Plot 1 (see report for 1931). Convert the corrected cuprous oxide to lactose (L), using the sugar table marked "Lactose" (Table 9)[1]. The percentage of lactose is then obtained from the following relationship:

$$\text{Percentage lactose} = \frac{L(110+X)}{0.26 \ C},$$

in which X is the value obtained in the polarimetric sucrose determination and C is the volume of solution in cc. used in the above lactose determination.

RECOMMENDATIONS[2]

It is recommended—

(1) That the proposed method for the determination of sucrose in chocolate be adopted as a tentative method.

(2) That the proposed method for the determination of lactose in milk chocolate be adopted as a tentative method.

(3) That the present tentative methods for the determinations of sucrose and lactose be dropped.

(4) That further collaborative work be done on the proposed methods with the view to adopting them as official.

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[1] *Methods of Analysis, A.O.A.C.*, 1930, p. 514.
[2] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 67 (1933).

REPORT ON COFFEE

By E. M. BAILEY, *Referee* and W. J. MATHIS (Connecticut Agricultural Experiment Station, New Haven, Conn.)

Both of the methods for the determination of caffeine in coffee now recognized in the official methods of this Association give caffeine residues of a high degree of purity. Twenty market samples of coffee examined in the referee's laboratory[1] showed that the average caffeine content as determined by the Fendler-Stüber method on the basis of the weight of the caffeine residue was 1.33 per cent and that the caffeine estimated from the nitrogen content of the residue averaged about 0.14 per cent lower. In 10 of the samples the variation between the two methods of evaluating caffeine did not exceed 0.1 per cent and in only 4 samples did it exceed 0.2 per cent. As the magnitude of the variations in exceptional cases may be as much as 20 per cent of the total caffeine present it is advisable to test the purity of the caffeine residue by a nitrogen determination in all cases, and the official procedures now make such a precaution mandatory.

In coffees that have been processed to remove caffeine and that may contain only 0.2 per cent or less of that substance, obviously the impurity unavoidably present in the caffeine residue will comprise a correspondingly greater proportion of the total caffeine present. A satisfactory estimate of caffeine in such cases can only be made when it is based upon a determination of nitrogen in the caffeine residue. Clifford[2] studied methods for the determination of nitrogen in caffeine residues obtained from low caffeine (or so-called "decaffeinated") coffees. He concludes that caffeine may be estimated with sufficient accuracy on the basis of nitrogen determination by micro methods, or, if suitable precautions are taken, by ordinary methods. By a micro method and the use of Pregl tubes for digestion and a modified Parnas-Wagner apparatus for distillation, caffeine determined in four residues obtained by the Power-Chesnut method gave an average value of 0.0209 expressed in percentage. Because this procedure gave a recovery of 2.01 mg. of caffeine as an average for five determinations when a known quantity (2.0 mg.) was present, Clifford regards this procedure as yielding values that may be regarded as exact. Apparently some difficulty may be encountered in carrying out the technic of the sublimation process as applied to caffeine residues and this offsets the advantage of its greater theoretical accuracy.

Allen[3] studied methods for the determination of small amounts of caffeine and reached essentially the same conclusions as were reported by Clifford. Both investigators agree that the ordinary Kjeldahl procedure with some modifications will give sufficiently accurate results.

[1] *Connecticut Agr. Expt. Sta. Bull.* 276, p. 338 (1925).
[2] *This Journal*, 14, 533 (1931).
[3] *Ibid.*, 13, 265 (1930).

There is an obvious advantage from the standpoint of time and convenience if ordinary methods will suffice, therefore the work done this year was directed to prove their reliability. Reagents used in digestion were reduced to one-half the quantities ordinarily used and the same distillation apparatus as ordinarily employed for nitrogen determinations was used, but precaution was taken to steam the tubes thoroughly immediately before distillations were made. The usual 0.1 N solutions instead of more dilute solutions were used for titrations. The experimental material was pure anhydrous caffeine and blanks were run on reagents, pure sucrose being substituted for caffeine. The charge of caffeine taken for nitrogen determinations was 0.00391 gram, which on a 20 gram basis (combining the residues from duplicate determinations each on 10 grams of coffee) represents 0.0196 per cent of caffeine. The minimum percentage recovery in eleven trials was 0.0184 per cent; the maximum, 0.0230 per cent, and the average was 0.0207 per cent. These recoveries do not equal the order of accuracy reported by Allen[1] who, working on a charge of 0.00202 gram of caffeine, recovered in six trials by a micro-Kjeldahl method a minimum of 0.00191 gram, a maximum of 0.00211 gram, and an average of 0.00203 gram. However, these results were obtained with very little modification of the regular procedure for nitrogen determinations. Moreover, in practice there appears to be little occasion to express results in percentages beyond the second place of decimals, but assuming that the third place may be significant, the referee's variations from a theoretical value of 0.020 per cent are from -0.002 per cent to $+0.003$ per cent, and the average is $+0.001$ per cent.

Pending collaborative experience and comment on the procedure used in the referee's laboratory no method is recommended for adoption at this time, and it is therefore recommended[2] that the study be continued during the coming year.

No report on gums in foods was given by the referee.

REPORT ON FATS AND OILS

By GEORGE S. JAMIESON (Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

It was impossible for the referee to make the collaborative study recommended in the previous report on the application of the Albert method to the determination of the acid and saponification values of dark-colored fats and oils. However, some study was made in the referee's laboratory on the application of the method to the determination of the

[1] *Loc. cit.*
[2] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 67 (1933).

acidity of the oil extracted from cottonseed. The results were consistently lower than those obtained with the Association's official procedure. In view of these results and similar results reported by another laboratory, no further study of the Albert method is planned for the coming year.

It is recommended that a study be made of the standard methods for the analysis of cottonseed[1] with a view to their adoption by the Association. For several years the referee's laboratory has been engaged in an extensive investigation of such methods and has participated for two years in a collaborative study conducted by the National Cottonseed Products Association.

RECOMMENDATIONS[2]

It is recommended—

(1) That the vacuum oven method for the determination of moisture in fats and oils be made official (final action).

(2) That a collaborative study be undertaken of methods for the determination of the oil content of flaxseed and that I. H. Hopper of the North Dakota Agricultural Experiment Station, Fargo, North Dakota, be appointed associate referee to take charge of this study.

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[1] U. S. Dept. Agr. Ser. Reg. Announcement 133 (June, 1932).
[2] For report of Subcommittee C and action of the Association, see *This Journal*, 16, 67 (1933).

WEDNESDAY—MORNING SESSION

SYMPOSIUM ON NEW ANALYTICAL METHODS

At the symposium on analytical methods held Wednesday morning the following papers were presented. References are given for those that have been published.

1. Microanalytical Methods, by E. P. Clark (*This Journal*, 16, 255 (1933)).
2. Solubilities of Some Organic Drugs and their Application in Drug Analysis, by L. E. Warren (see p. 571).
3. Determination of Fluorine, by O. B. Winter and H. J. Wichmann (*This Journal*, 16, 105 (1933)).
4. Apparatus for Measuring Turbidity and Color Intensity, by R. A. Osborn (to be published).
5. A Study of the Accuracy of the Kjeldahl Method, I. Use of Mercury as a Catalyst, by R. A. Osborn and Alexander Krasnitz (*This Journal*, 16, 107 (1933)).
6. Analytical Tolerances, by R. H. Kerr (paper not presented).
7. Determination of Selenium, by W. O. Robinson (*This Journal*, 16, 423 (1933)).
8. Analytical Difficulties Encountered in Mold Fermentation, by P. A. Wells (not published).
9. The Accuracy of Various Methods Used for the Determination of Butter Fat, by G. E. Holm (*J. Dairy Sci.*, 16, 445 (1933)).
10. Determination of the Susceptibility of Fats and Oils to Oxidation, by George R. Greenbank. (Résumé of work previously published.)
11. Arsenic in Plant Material, by R. B. Deemer and J. A. Schricker (*This Journal*, 16, 226 (1933)).
12. Modern Methods Used in the Manufacture and Distribution of Ice Cream, by O. E. Williams (not published).
13. Qualitative Test for Lead in Spray Residue, by M. Harris (*This Journal*, 16, 245 (1933)).

CORRECTION

Vol. XVI, p. 408.—In line 8 of reading matter change "53.111" to "53.106" and in line 10, formula, change $12H_2O$ to $2H_2O$.

CONTRIBUTED PAPERS

SOLUBILITIES OF SOME ORGANIC DRUGS AND THEIR APPLICATION IN DRUG ANALYSIS

By L. E. WARREN (U. S. Food and Drug Administration, Washington, D. C.)

The problem of separating and determining several organic substances in a mixture frequently confronts the drug analyst. If the substances are closely related chemically the separation may be difficult and, in the present state of knowledge, it is sometimes impossible. The Association of Official Agricultural Chemists early recognized the importance of the subject, and many valuable methods for the separation and determination of organic medicinal substances in mixtures have been elaborated by its members. However, with new mixtures of the older remedies constantly appearing and with the advent of new synthetic medicinals at frequent intervals, new analytical perplexities are almost constantly being encountered.

A number of years ago the writer had observed[1] that the literature did not give much specific information concerning the solubilities of synthetic organic drugs in the less-used solvents, such as benzol, carbon tetrachloride, and petroleum benzin. The same situation existed concerning many plant constituents. It seemed reasonable to suppose that if more information of this sort were available approximate separations of certain organic substances that are not now possible might be made. At that time the writer also suggested that more data in these directions should be given in the Pharmacopoeia of the United States. He began to collect information on the subject and to make solubility determinations as opportunity offered. Later he enlisted the services of a number of students and instructors in pharmaceutical schools, as well as others. The information desired is by no means complete.

A list of the individual organic substances that had considerable use in medicine, including organic bases, was first prepared. The salts of these bases are excluded because of their small solubilities in organic solvents. The solubilities of the listed substances in benzol, carbon tetrachloride, and petroleum benzin were tabulated so far as such facts could be obtained by moderately complete search of the literature. The information available was meager, and in many instances that found was conflicting.

Determinations of the solubilities in the solvents mentioned were then made by the writer and his collaborators. The method used is essentially the same as that described in the U.S.P.X.[2] In the case of easily volatile substances precautions were taken to prevent losses during the drying

[1] J. Am. Pharm. Assoc., 13, 133 (1924).
[2] Pharmacopoeia of the United States of America, 10, 458 (1925).

of the residues before weighing. The findings are given in Table 1, the drugs being arranged in alphabetical order. In the instances involving no experimentation information from the literature is included. It will be noted that many statements such as "soluble," "easily soluble," etc., are recorded. These indicate the need for continued study.

TABLE 1

Solubilities of some organic substances in certain organic solvents
arranged alphabetically
(Gram per 100 grams.)

SUBSTANCE	SOLVENT		
	BENZENE	CARBON TETRACHLORIDE	PETROLEUM BENZIN
Acetanilid	2.46	0.102	0.03
		0.120 0.102	
Acetphenetidin	0.65	0.037	0.015
Acetylsalicylic Acid	0.325	0.04	No weighable residue
Amidopyrine	29.842	8.8	0.648
	24.468		0.647
Antipyrine	8.105	1.031	No weighable residue
Atropine	3.992	0.661	0.083
Barbital	0.057	0.007	No weighable residue
		0.009	
Beta-naphthol	4.13	0.442	No weighable residue
Brucine	0.111	0.078	0.088
Caffeine	0.91	0.09	Somewhat soluble
		0.26	(Slight)
Chrysarobin	4.0	Soluble	Somewhat soluble
Cinchonidine	0.099	0.051	0.048
Cinchonine	0.055	0.044	0.032
Cinchophen	0.026	0.0035	Insoluble
	0.027	0.0037	
Cocaine	100.0	100.0	2.37
		31.94	
Codeine	14.83 8.707	3.882	0.170 0.189
	14.88 11.400	1.328	0.140 0.228
Hydrastine	8.889	0.123	0.073
Morphine	0.0623	0.0250	0.0854
		0.0156	
Narcotine	4.729	0.911	0.025
	4.688	0.909	0.019
	4.000		0.027
Phenobarbital	Somewhat soluble	Soluble	(0.001) 0.0098
			0.0069
Phenolphthalein	0.16	0.001	0.018
		0.004	0.017
			0.021
Quinidine	2.45	0.564	0.024
Quinine	0.56	0.54	0.024
	1.7	0.53	0.021

SUBSTANCE	SOLVENT		
	BENZENE	CARBON TETRACHLORIDE	PETROLEUM BENZIN
Quinine ethylcarbonate	29.842	Soluble	1.219
			1.217
Resorcinol	3.18	Insoluble	0.003
			0.003
Salicyclic acid	0.846	Slightly soluble	Very slightly soluble
Salol	229*	285*	91*
	88.57		
Santonin	5.233	0.302	0.012
	5.184	0.307	0.0212 0.011
Strychnine	0.667	0.26	0.009
	0.679		
Sulfonal	0.76	0.90	Slightly soluble
Theobromine	0.005	0.02	Practically insoluble
Trional	Soluble	Very soluble	0.400
			0.420

* Approximate

In order to make the information more readily practicable, the table of findings was rearranged in the approximate order of solubilities, those drugs having the least solubilities being given first.

The solubilities of those drugs (in couplets) which are occasionally prescribed together, such as acetphenetidin and salol, acetanilid and salol, etc., were also compared by selecting from Table 1, ten drugs, the solubilities of which (when the drugs are arranged in couplets) present sufficient differential to suggest a possible method of separation. These are arranged in Table 3. Accordingly, in Table 3 the solubilities of any given drug should be compared with those of the drug immediately above or directly below it.

If these principles of differential solubility are applied to the approximate separation of mixtures, it must be remembered, of course, that the laws of solubility for single substances no longer strictly apply. If two substances (for example salol and acetphenetidin) be treated with a solvent (for example, petroleum benzin) in which the salol is readily soluble and in which the acetphenetidin is but scantily so, the true solubility of the acetphenetidin in this solvent is the solubility of acetphenetidin in petroleum benzin containing the quantity of salol known to be present.

ADDITIONAL REFERENCES

Henry. The Plant Alkaloids, 1924.
Seidell. Solubilities of Inorganic and Organic Compounds, Vol. 1, 1919; Vol. 2, 1928.
Allen. Commercial Organic Analysis, 5th ed., Vol. 7 (1929).
Coutts. *Pharm. J.*, **128**, 262 (1932).

TABLE 2

Solubilities of some organic substances in certain organic solvents arranged in the relative order of their solubilities

(Gram per 100 grams.)

SUBSTANCE	BENZENE	SOLVENT CARBON TETRACHLORIDE	PETROLEUM ETHER
Cinchophen	0.026 0.027	0.0035 0.0037	Insoluble
Barbital	0.057	0.007 0.009	No weighable residue
Theobromine	0.005	0.02	Practically insoluble
Acetylsalicyclic Acid	0.325	0.04	No weighable residue
Beta-naphthol	4.13	0.442	No weighable residue
Antipyrine	8.105	1.031	No weighable residue
Salicyclic acid	0.846	Slightly soluble	Very slightly soluble
Caffeine	0.91	0.09 0.26	Somewhat soluble (Slight)
Chrysarobin	4.0	Soluble	Somewhat soluble
Sulfonal	0.76	0.9	Slightly soluble
Resorcinol	3.18	Insoluble	0.003 0.003
Strychnine	0.667 0.679	0.26	0.009
Phenobarbital	Somewhat soluble	Soluble (0.001)	0.01 0.007
Acetphenetidin	0.65	0.037	0.015
Phenolphthalein	0.16	0.001 0.004	0.017 0.021
Santonin	5.233 5.184	0.302 0.307	0.011 0.012
Narcotine	4.729 4.688 4.0	0.911 0.909	0.019 0.027 0.025
Quinine	0.56 1.7	0.54 0.53	0.024
Quinidine	2.45	0.564	0.024
Acetanilid	2.46	0.102 0.120 0.102	0.03
Cinchonine	0.055	0.044	0.032
Cinchonidine	0.099	0.051	0.048
Hydrastine	8.889	0.123	0.073
Atropine	3.992	0.661	0.083
Brucine	0.111	0.078	0.088
Morphine	0.0625	0.0250 0.0156	0.0854
Codeine	14.88 14.83 8.707 11.4000	3.882 1.328	0.170 0.189 0.140 0.228
Trional	Soluble	Very soluble	0.400 0.420
Amidopyrine	29.843 24.468	8.8	0.648 0.647
Quinine ethylcarbonate	29.842	Soluble	1.219 1.217
Cocaine	100.0	100.0 31.94	2.37
Salol	229* 88.57	285*	91*

TABLE 3
Solubilities of some organic substances in certain organic solvents arranged in couplets
(Gram per 100 grams.)

SUBSTANCE	BENZENE	CARBON TETRACHLORIDE	PETROLEUM BENZIN
Acetylsalicyclic Acid	0.325	0.040	Unweighable
		0.102	
Acetanilid	2.46	0.102	0.03
		0.120	
Salol	229.0*	285.0*	91.0*
Acetphenetidin (Phenacetin)	0.065	0.037	0.015
Amidopyrine	29.842	8.8	0.648
	24.468		0.647
Barbital	0.057	0.009	Unweighable
		0.007	
Cocaine	100.0	100.0	2.37
		31.94	
Quinine	1.7	0.54	0.024
	0.56	0.53	0.021
Phenolphthalein	0.16	0.001	0.021
		0.004	0.017
			0.018
Santonin	5.233	0.307	0.011
	5.184	0.302	0.012
			0.J21

* Approximate.

Utz. *Süddeut. Apoth. Ztg.*, **60**, 430, 442 (1920); *C.A.*, **15**, 1236 (1921).
Langer. *Apoth. Ztg.*, **43**, 815 (1928).
Müller. *Apoth. Ztg.*, **18**, 257–266 (1903).

SEMIMICRO DETERMINATION OF NITROGEN BY THE DUMAS METHOD

By E. P. CLARK (Insecticide Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.)

In further amplification of the paper on semimicro organic analyses presented at the November, 1932, meeting of the A.O.A.C.[1] the following

[1] Clark, *This Journal*, **16**, 256 (1933).

procedure for the determination of nitrogen by the Dumas method is given. The principle involved is that of burning a substance with the aid of cupric oxide in an atmosphere of pure carbon dioxide and collecting and measuring over strong potassium hydroxide solution the nitrogen thus formed. The apparatus required for these operations consists of a carbon dioxide generator, a combustion furnace with a properly filled tube and an azotometer to collect the nitrogen. The equipment recommended is shown schematically in the accompanying drawing.

Carbon Dioxide Generator.—Several good carbon dioxide generators have recently been described,[1] but from the standpoint of simplicity of construction and operation the one shown in the drawing is preferred. The carbon dioxide is formed by the action of dilute hydrochloric acid upon prepared marble. Clean dense marble, broken into pieces that will pass through the ground joint (7), is immersed in water in a beaker covered with a watch-glass, and alternately boiled and cooled for several days. It is then placed in (6), the ground joint (7) is sealed with glyptol resin,[2] (8) is closed and (5) is three-fourths filled with approximately 20 per cent hydrochloric acid. A few small pieces of marble are dropped through (4) to permit the evolved carbon dioxide to help sweep the acid free of air. The mercury reservoir (2) is connected with (5) through (4) by means of prepared rubber tubing.* The ends of the glass tubing at this connection, as well as at (9) and (10), should be flush with each other and covered with cellogrease (or a similar stopcock lubricant). The cocks (1), (3) and (8) should be equipped with stopcock clamps as shown.[3]

When the apparatus is assembled (1) and (8) are closed and an oil vacuum pump is connected to (3) and (8, closed). At first the evacuation through (3) must be carefully controlled with a screw pinch clamp, but after a good vacuum is attained the clamp is removed. After the system has been pumped for 10 minutes (3) is closed and (8) is opened, which causes the acid to rise in (6) to the marble. The vacuum connection to (8) is closed for a short time by pinching the rubber connection to the pump and then suddenly releasing and closing it in such a way that the acid surges onto the marble and generates sufficient carbon dioxide to cause some of it to pass out of (6) through (5) and into (2). At first, however, care must be taken that the reaction is not too violent. Several repetitions of this process will cause sufficient carbon dioxide to accumulate in (2) to produce a pressure of approximately 10 cm. of mercury (controlled by the leveling bulb). In filling (2), (1) is cracked from time to time to determine by means of the flow of the mercury the pressure in B. When

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[1] Path, *Ind. Eng. Chem. Anal. Ed.*, **3**, 202 (1931); Trauts and Niederl, *Ibid.*, 151; and Lowe and Guthmann, *Ibid.*, **4**, 440 (1932).
[2] Sager and Kennedy, Jr., *Physics*, **1**, 352 (1931).
* Short sections of good grade antimony (red) rubber tubing are immersed in molten paraffin contained in a round-bottomed flask heated on a steam bath. The system is evacuated with an oil pump until gas ceases to be evolved, after which the vacuum is removed. The flask is then left on the steam bath for another half hour, when the tubing is removed and thoroughly wiped inside and out. Rubber tubing thus treated and used for connections will not cause contamination of pure carbon dioxide.
[3] See Roe, *Science*, **77**, 566 (1933).

atmospheric pressure is attained, A is opened. The bubble counter and connection (10) leading to the combustion tube is then attached.

Tube Filling.—It is desirable to abandon the classical procedure of emptying and refilling the combustion tube after each determination and instead proceed as in the determination of carbon and hydrogen. For this purpose the tube filling shown in the diagram has been adopted. The combustion tubing is the same as that recommended for the determination of carbon and hydrogen, and the size of the fillings for the various units conform to the dimensions of the combustion furnace previously described.[1] Before new copper oxide wire is used it should be ignited in oxygen for at least 2 hours and then allowed to cool in a stream of carbon dioxide. The reduced copper coil is prepared by heating it to redness and while at this temperature placing it quickly in a test tube containing about 1/2 cc. of pure methanol. The alcohol immediately ignites, and for the most part is consumed. As soon as the flame recedes within the tube the latter is connected to a vacuum, and the whole system is allowed to cool and dry under reduced pressure. The coil is then ready to place in the combustion tube. The cartridge element, which contains the sample and fine copper oxide to supply the necessary oxygen for the combustion, is the device recommended for this purpose by Couch[2] and is superior from the standpoint of convenience and efficiency to any other known arrangement.

Azotometer.—This part of the apparatus may be made from a 10 cc. Normax buret, the stopcock of which is removed and sealed to the top end of the instrument. The blank space between the graduations and the cock should be roughly 0.5 cc. The buret tip is removed, and the graduated portion is sealed at the 5 cc. mark to the large section of the apparatus. The graduated portion and the blank space between the cock and the zero mark are then accurately calibrated. Such an apparatus is easily read to 0.01 cc.

Mercury is placed in the lower part of the instrument so that its level is 5 mm. above the gas inlet side tube, and the rest of the azotometer is filled through the leveling bulb with Pregl's so-called 50 per cent potassium hydroxide solution.[3]

The Combustion.—When the apparatus is assembled as outlined, 15 to 25 mg. of substance, depending upon its nitrogen content, is weighed and mixed in a 10 cc. beaker with fine copper oxide powder which has previously been ignited and cooled in a stream of carbon dioxide. (It is convenient to preserve the copper oxide in a stoppered test tube placed in a fruit jar or similar container filled with carbon dioxide.) The cartridge is filled as indicated in the diagram, first with copper oxide powder, then

[1] Clark, *This Journal*, 16, 414 (1933).
[2] *J. Am. Chem. Soc.*, 55, 852 (1933).
[3] Pregl. Quantitative Organic Microanalysis. Translated by Fylman. Blakiston's Sons Co., Philadelphia (1930), page 99.

with the oxide containing the substance, and finally with the oxide used to wash out the beaker. A measure is recommended to dispense the necessary quantities of the oxide. When the cartridge has been properly filled, the coil in section B of the furnace is removed, the cartridge is inserted and the coil is replaced. With the permanent and removable filling (Section D) already in place the combustion tube is connected to the carbon

SEMIMICRO DUMAS NITROGEN APPARATUS

U S DEPARTMENT OF AGRICULTURE
BUREAU OF CHEMISTRY AND SOILS
INSECTICIDE DIVISION
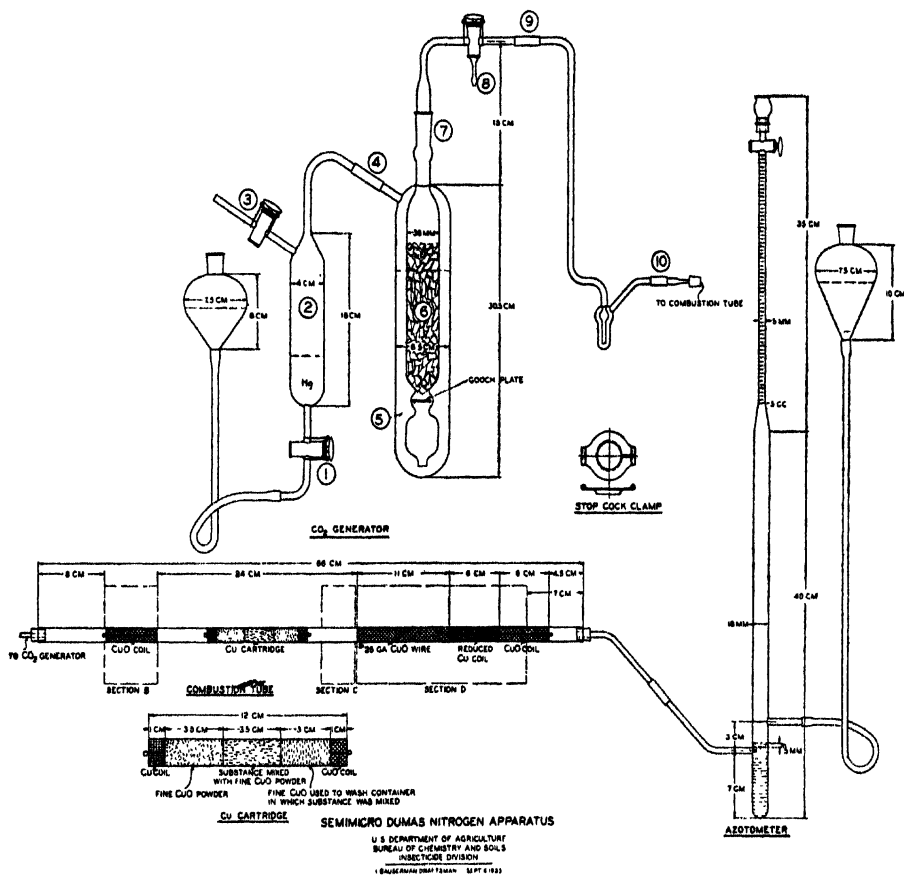
dioxide generator and the azotometer. The potassium hydroxide is removed from the azotometer by lowering the leveling bulb and opening the stopcock. A fairly rapid stream of carbon dioxide is then passed through the system, and at the same time heat is applied to sections B, C and D of the furnace. (The position of B and C at this stage is that shown in the diagram.) As soon as a dull red heat is attained in these sections the potassium hydroxide solution is returned to the azotometer and it is observed whether the residual bubbles from the absorption of the carbon dioxide are of pin-point size; if not, the system requires further

sweeping out, but if they are, the azotometer is completely filled with the potassium hydroxide solution, including some in the reservoir above the stopcock. The rate of flow of the carbon dioxide is reduced to approximately 1.5 cc. per minute and the heating elements B and C are moved toward the cartridge. As the latter and its contents become sufficiently hot combustion of the substance begins, as is indicated by a more rapid flow of carbon dioxide into the azotometer. The burning, controlled by the rate at which the elements B and C are caused to approach each other, must be such that a slow even stream of gas is evolved. Approximately twice as many bubbles as come from the generator is a desirable speed, and at such a rate approximately 20 minutes is required for the actual combustion.

Both burners are advanced toward each other at the same rate until the tip of the cartridge under section B is at a dull red heat. Thereafter only C is advanced toward the left.

If the materials are volatile or easily decomposed it may not be possible to heat the cartridge as much as indicated. In such cases the procedure must be governed entirely by the rate at which the material burns.

When the combustion in the cartridge is completed the rate of flow of the bubbles passing into the azotometer reassumes that set by the generator. C may then be moved more rapidly toward B. When the two burners meet, C is brought back 1 cm. at a time to its original position. Coincident with this operation the flow of carbon dioxide from the generator is approximately doubled until the diameter of the bubbles is less than 1 mm. The heating in all units is then discontinued, and a fairly rapid stream of carbon dioxide is passed through the tube in order to sweep all the nitrogen into the azotometer. When the residual bubbles become pin point in size, as in the beginning of the operation, the procedure is completed. The azotometer is disconnected and after a few minutes the volume of gas is read.

In calculating the percentage of nitrogen in the sample the following formula is used:

$$\text{Wt. of nitrogen in mg.} = \frac{1.2507\ (V)\ (P-P')\ (273)}{(760)\ (273+t)}$$
$$= \frac{(0.4493)\ (V)\ (P-P')}{(273+t)},$$

where V = the corrected azotometer reading; P = the barometric pressure, P' = the aqueous tension of the KOH solution used; and t the temperature of the gas.

The values for P' for Pregl's KOH solution are approximately as follows:*

t	P' in mm.	t	P' in mm.
15	5.5	26	9.3
16	5.7	27	9.8
17	6.0	28	10.4
18	6.4	29	10.9
19	6.7	30	11.4
20	7.0	31	12.0
21	7.3	32	12.6
22	7.6	33	13.1
23	8.0	34	13.6
24	8.4	35	14.0
25	8.9		

* The solution upon which these values were obtained was made according to Pregl's directions from a good C.P. grade of KOH, containing 16.5 per cent moisture. 100 cc. of the solution contained 61.5 gram of KOH.

In the usual micro Dumas method several corrections are applied to the nitrogen volume as read. As indicated by Trautz[1] these are: (1) the occluded air in the temporary filling, (2) the wall error of the azotometer, (3) the contamination of the CO_2, and (4) the vapor pressure of the KOH solution. In the procedure outlined only the first of these is significant. The copper oxide powder used to fill the cartridge when prepared and stored as recommended usually contains approximately 0.04 cc. of occluded gas not absorbed by the KOH solution. This value, however, should be determined for each lot of reagent, and also on the same lot after an appreciable interval of time. This is done by burning a sample of nitrogen-free substance under the conditions governing a regular combustion. Concerning the other three factors enumerated it has been found that an azotometer of the size described has, after 15 minutes, no measurable wall error; the carbon dioxide from the generator assembled as outlined is of such purity that no measurable residual gas is formed during a combustion; and finally, the formula suggested for calculating the nitrogen formed involves the correction for the vapor pressure of the KOH solution.

A few analyses made by the method as outlined are presented in the following table:

SUBSTANCE	SAMPLE	V CORRECTED	t	P	NITROGEN FOUND	NITROGEN CALCULATED
	mg.	cc.	°C.	mm.	per cent	per cent
α-Nitronaphthalene	26.28	1.92	32	759	8.03	8.05
γ, γ-Dipyridyl dihydrate	26.84	3.56	30	760	14.72	14.55
Acetanilid	25.67	2.39	30	765	10.40	10.37
Phenylglucosazone	20.93	2.91	30	763	15.53	15.64
2, 6-Dimethoxybenzonitrile	22.07	1.67	30	763	8.43	8.59
Gossypol dianilide	21.93	0.81	30	762	4.11	4.19

[1] *Microchemie*, 9, 300 (1931).

IDENTIFICATION OF FLAVORING CONSTITUENTS OF COMMERCIAL FLAVORS[1]

IV. IDENTIFICATION OF BENZOIC ACID

By JOHN B. WILSON and GEORGE L. KEENAN

In testing for γ-undecalactone[2] in the various fractions obtained in the qualitative separation of classes[3] of chemical compounds present in commercial flavors and in known mixtures used to to test the method, it was found that fraction B of the nonvolatile constituents gave a crystalline substance with hydrazine hydrate when no γ-undecalactone was present. As the samples were known to contain sodium benzoate, it was suspected that this crystalline substance was formed from the benzoic acid extracted from the non-volatile portion of the samples.

Accordingly, 0.5 gram of benzoic acid was treated with hydrazine hydrate as described in Part III.[2] After evaporation on the steam bath it was found that instead of the thick liquid which remains in the case of γ-undecalactone, solid matter remained in the beaker after all the water had evaporated and the ammoniacal odor was no longer perceptible. These crystals, which had a melting point of 112° C., were examined microscopically and found to have the following properties:

OPTICAL PROPERTIES OF BENZHYDRAZIDE

The substance is essentially colorless when examined in ordinary light under the microscope and consists of irregular fragments without definite habit. In parallel polarized light (crossed nicols) in addition to the fragments which extinguish sharply, there are those which remain essentially bright when the stage is rotated under the same conditions. In convergent polarized light (crossed nicols), the fragments which do not extinguish sharply show a partial biaxial interference figure with one optic axis up or slightly inclined. The refractive indices as determined by the immersion method are as follows: $\alpha = 1.545$ (common); $n_s = 1.567$ (most common of the indices and closely approximating the β-value); $\gamma = 1.685$; all ± 0.003.

As the literature was found to contain no instance of benzhydrazide having been prepared from the direct union of hydrazine hydrate and benzoic acid, a quantity of this substance was made in the usual manner by refluxing a mixture of methyl benzoate and hydrazine hydrate, and the crystals obtained were identical with those obtained from the acid. After several trials it was found that a form of crystal more satisfactory for microscopical work was obtained by the evaporation of the aqueous mixture of benzoic acid and hydrazine hydrate than by recrystallization of the residue from ethyl or butyl alcohol. It was found, however, that

satisfactory crystals were obtained by dissolving the residue from the aqueous solution in warm ether, followed by the spontaneous evaporation of the ether. These crystals also melted at 112° C. and had the optical properties given above.

HYDRAZIDE TEST FOR BENZOIC ACID (QUALITATIVE)

To fraction B obtained by carrying out the procedure for the qualitative separation of classes[1] or other extracted matter suspected to contain benzoic acid, add a slight excess of hydrazine hydrate solution (40 per cent in water). Mix well and allow to stand at room temperature for 15–20 minutes. Evaporate on the steam bath until dry or until the ammoniacal odor is no longer perceptible. The presence of benzoic acid in the fraction tested is shown by the formation of crystals of benzhydrazide. Establish the identity of the crystals by examination under the microscope according to the optical-immersion method. To obtain larger crystals, dissolve the residue in warm ether, filter off any insoluble matter, and permit the filtrate to evaporate spontaneously. (Benzhydrazide appears to decompose after a few months as it becomes discolored and its optical properties are markedly altered. Therefore reference samples are valueless if discolored and should be replaced by freshly prepared material.)

Interfering substances (cinnamic, anisic, stearic, palmitic and myristic acids; vanillin, coumarin, and heliotropine), which it was thought might be found in fraction B under practical application of the separation were subjected to the test and were found to give no crystals.

SUMMARY

Benzhydrazide was prepared from benzoic acid, and an ester of that acid and its optical properties were ascertained by the immersion method.

A qualitative test is given whereby benzoic acid may be identified in flavors, etc. The benzoic acid is converted to benzhydrazide with the subsequent identification of the latter by examination under the microscope according to the optical-immersion method.

COMPARATIVE STUDY OF THE PAIRED AND AD LIBITUM FEEDING METHODS FOR DETERMINING AMINO ACID DEFICIENCIES OF FEEDS

By C. L. SHREWSBURY and J. W. BRATZLER*
(Departments of State Chemist and Animal Husbandry, Purdue University, Agricultural Experiment Station, Lafayette, Indiana)

In discussing the essentials of a good nutrition experiment Mitchell[2] points out the importance of equalized feed consumption of experimental

animals. In a study of the fundamental food requirements for growth of the white rat Palmer[1] emphasizes the part played by the efficiency quotient in the growth of the animal. In the majority of nutrition experiments reported in the literature the ad libitum method of feeding has been used. So far as the writers have determined, no subject in nutrition has been investigated in which, simultaneously and under the same laboratory conditions, both the ad libitum and the paired methods of feeding have been used.

Experiments were therefore designed to test the relative accuracy of these two experimental procedures by an examination of the alleged deficiency of soybean protein in the amino acid cystine.

EXPERIMENTAL PROCEDURE

Three rations were used in the experiments. One contained 10 per cent soybean protein in a purified diet, another 15 per cent protein, and a third contained 6 per cent soybean protein as a supplement to corn. The rations were fed to rats with and without the addition of cystine. In one series feed consumption was equalized (paired feeding method); in the other the animals were allowed to eat ad libitum. In both series the animals were separated into pairs. The animals of each pair were selected from stock at 3 weeks of age, were of the same age and sex, were similar in weight, and were from the same litter. Thus the only known variable in the two series was feed consumption.

TABLE 1

Statistical values from experiments with rats in which feed
consumption was equalized and ad libitum

	SOYBEAN PROTEIN 10 PER CENT		SOYBEAN PROTEIN 15 PER CENT		CORN, SOYBEAN PROTEIN	
	FEED EQUALIZED	FEED AD LIBITUM	FEED EQUALIZED	FEED AD LIBITUM	FEED EQUALIZED	FEED AD LIBITUM
Total mean difference in weight, M	+9.6[a]	+11.1[a]	+12.1[a]	+10.5[a]	+0.17[a]	−9 9[b]
Standard Deviation, S	4.95	12.5	6.31	14.1	6.03	21.4
Ratio M:S, Z	1.97	0 88	1.93	0 77	0 028	0 46
Probability, P	0.0006	0.026	0.0007	0 041	—[c]	0 1332
Probable Error	±1.17	±3.94	±1.48	±3.32	± .943	±5 0
No. of Experiments	8	8	8	8	18	8
No. of Animals on Experiment	16	16	16	16	36	16

[a] Total mean difference in weight favors cystine supplemented ration.
[b] Total mean difference in weight favors control ration.
[c] Student's tables do not record a probability for a value of Z smaller than 0.1.

EXPERIMENTAL RESULTS

The results of all the trials have been summarized statistically in Table 1 according to the method of Student.[2] The total mean difference in weight between the cystine supplemented rations and the unsupplemented control rations is shown. The degree of probability that the result is due to chance is derived from the mean difference (M) and the standard

[1] *J. Biol. Chem.*, **90**, 545 (1931).
[2] *Biometrika*, **6**, 1 (1908).

deviation with the aid of tables also prepared by Student. If P is smaller than 0.02 it can be assumed that chance has not played an important part in the final result. The probable error of the experiments, determined in the regular way, is also recorded.

Striking differences in the accuracy of the two methods of feeding are shown. In connection with the data on the ration containing 10 per cent of soybean protein the probability that the difference in gain in favor of the cystine supplemented ration was due to chance was very small when feed consumption was equalized ($P = 0.0006$). The probable error of the result (± 1.17) is also quite small. In the ad libitum experiments the probability that the result was due to chance was larger ($P = 0.026$). The probable error was also larger (± 3.94).

In the experiments with 15 per cent soybean protein the statistical data reveal that the series in which feed consumption was equalized gave the most reliable results. The probable error of the mean result was higher and the probability that the result was due to chance was also higher in the ad libitum series of experiments.

In the experiments with soybean protein as a supplement to corn and where no cystine deficiency can be said to exist the statistical results again show the greater reliability of the series in which food consumption was equalized.

It would seem from these experiments that where unequal feed consumption may produce irregular gains a larger number of experiments would be required to establish a given result.

SUMMARY

Data calculated statistically according to the method of Student, from nutrition experiments with rats in which the paired and ad libitum feeding methods were employed indicate that the paired feeding method, in which feed consumption was equalized, gives the most trustworthy results.

A STUDY OF THE SCHMID-BONDZYNSKI METHOD FOR THE DETERMINATION OF FAT IN CHEESE

By HENRY A. LEPPER and LESLIE HART (Food Control Laboratory,[1] Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

In 1888 Werner Schmid[2] proposed a method for the estimation of the fat content of milk and cream involving heating with concentrated hydrochloric acid and extraction with ethyl ether, the fat being weighed as the

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[1] W. B. White, Chief.
[2] *Z. anal. Chem.*, **27**, 464 (1888).

residue from an aliquot taken from the measured total ether layer. The next year Stan. Bondzynski[1] modified the graduated cylinder to facilitate measuring the ether layer and the withdrawal of the aliquot. In 1894 Stef. Bondzynski[2] applied the method to cheese. The method, in essentially this form, was used by Kirsten,[3] who obtained closely agreeing results on numerous varieties of cheese. Ratzlaff[4] introduced the use of mixed ethers, ethyl and petroleum, making only one extraction in a straight graduated cylinder (Gottlieb tube). This method, modified to use the Röhrig tube, with three extractions with mixed ethers, was recommended by Patrick[5] in 1910 to the Association of Official Agricultural Chemists, and it is essentially the method now appearing in *Methods of Analysis, A.O.A.C.*, 1930, 239.

No record of collaborative study made at the time of adoption could be found. The method was adopted as tentative in 1916.[6] Hortvet[7] reported what appears to be the only collaborative work with the method in 1917, and as a result it was adopted as official, first action, that year.[8] Without further collaborative study or recommendation by the referee it was adopted as official, second action, in 1921.[9] Hortvet,[10] in 1925, apparently without realization of this action, states: "The Schmid-Bondzynski method, modified, still appears, in the experience of the referee and in the judgment of a number of collaborators, reasonably satisfactory and capable of giving good results. It does not seem, however, that experience with this method will justify any recommendation except that it remain in its present form as tentative." Borello,[11] after investigating various modifications of the method and comparing them with the Soxhlet, Gerber, Roese-Gottlieb and Smetham methods, concluded that for highest accuracy the Schmid-Bondzynski procedure was to be preferred. This procedure differs principally from the present method in that ethyl ether only is used.

Although official, the present method did not receive the close scrutiny usually afforded by the Association. This fact, together with the doubt expressed by Hortvet as to its complete suitability, led to this study of the method, which was designed to show that different analysts working on the same samples can get concordant results.

EXPERIMENTAL

In order to eliminate the factor of sampling a weighed sample sufficient for an analysis was sent to each collaborator in a container that could be

[1] *Landw. Jahrb. Schweiz.*, 3, 119 (1889).
[2] *Z. anal. Chem.*, 33, 186 (1894).
[3] *Z. Unters. Nahr. Genussm.*, 1, 742 (1898).
[4] Milch.-Ztg., 32, 65 (1903).
[5] Bur. Chem. Bull. 137, 169 (1911).
[6] *This Journal*, 3, 534 (1920).
[7] *Ibid.*, 4, 201 (1920).
[8] *Ibid.*, 248.
[9] *Ibid.*, 5, 136 (1922).
[10] *Ibid.*, 8, 475 (1925).
[11] *Ann. stas. sper. Caseificio Lodi*, p. 23 (1919).

used, without transfer of the cheese, for the acid digestion previous to the fat extraction. Tall-form lipless beakers, 1½×4 inches, were made from Pyrex test tubes and fitted with cork stoppers covered with tin foil. Preliminary tests gave results for fat (moisture-free basis) on a cheese in three beakers of 46.34, 46.31 and 46.27 per cent. The moisture of the cheese determined at the time the beakers were filled was 28.03, 27.97 and 27.97 per cent. The fat at that time was 46.30 and 46.31 per cent. These results showed that this manner of shipping the samples to collaborators would be satisfactory.

COLLABORATIVE STUDY

Two pounds of imported Swiss cheese was ground and thoroughly mixed, and a 1–2 gram charge was immediately weighed into each of twelve of the beakers. At the same time control samples were weighed out for immediate moisture and fat determinations. A 2-ounce sample was also transferred to each of twelve glass-topped, 4-ounce jars. The three series of samples were numbered uniformly. One beaker and one jar sample were sent to each collaborator, who was requested to determine fat only by following these directions:

Weigh the sample and container on an analytical balance. Shake down any particles of cheese from the sides and stopper and remove the stopper, transferring any adhering cheese into the container with a stirring rod, which is later used in filtering the ether solution of fat. Reserve the stopper for later treatment with fat solvents. Determine fat by the Schmid-Bondzynski method, using the container as the beaker for the acid digestion. (Separated fat, if any, need not be reincorporated before the digestion is begun.) When the ether and petroleum ether are added, pour them over the bottom of the stopper into the beaker to assure the inclusion of any fat that may adhere to the tin foil, taking care to obtain any fat adhering to the sides of the beaker. When the extraction is complete, remove the cheese residue from the digestion beaker, clean carefully, and air dry by allowing it to remain in the open air covered with a towel (not in a desiccator). (Do not clean the stopper further.) Air dry the stopper also and weigh the dry beaker and stopper together. In bringing the flasks containing the recovered fat, as well as the counterpoise flask, to constant weight, after removal from the oven, allow them to remain in the open air protected by a towel for 20 to 30 minutes before weighing and do not use a desiccator. Report gross weight of the sample and complete container, the tare weight of the container and stopper, and weight of recovered fat.

The collaborators were also directed to determine moisture by the method given in sections 90 and 91, p. 238, and fat by the Schmid-Bondzynski method, p. 239, *Methods of Analysis, A.O.A.C., 1930*, on the cheese in the glass-topped jars after through mixing to reincorporate any separated fat.

RESULTS

The results obtained on ten collaborative test-tube samples and on the corresponding control samples are given in Table 1. The results reported for a given sample are those obtained by a collaborator for fat by the

Schmid-Bondzynski method and those for moisture and fat as determined in the writers' laboratory on the control sample taken from the main batch immediately after the filling of the beaker with the collaborative sample. Despite the precautions taken a slight loss in weight occurred in the collaborative samples on shipment. The weights of the samples as placed in the beakers and those found on arrival are reported, and the fat is calculated on the basis of each weight. Closely agreeing fat results indicate that the losses were moisture. The fat results based on the weights put in are regarded as the more accurate as they agree more closely in each case with the fat in the respective control sample. The results on the control samples show that little or no change occurred in the main batch of comminuted cheese during preparation. All sampling was done in a 20°C. room to minimize loss of moisture.

TABLE 1

Results on weighed collaborative samples and corresponding control samples

SAMPLE NO.	COLLABORATIVE RESULTS				CONTROL RESULTS		
	WEIGHT SAMPLE		FAT		MOISTURE	FAT	
	PUT IN	ON ARRIVAL	PUT IN BASIS	ON ARRIVAL BASIS		ORIGINAL BASIS	DRY BASIS
	grams	*grams*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	2.090	2.0742	30.10	30.33	35.59	30.05	46.60
2	2.292	2.2493	30.38	30.96	35.69	29.97	46.60
3	2.300	2.3004	30.40	30.79	35.73	30.24	47.05
4*	1.836	1.8280	30.29	30.42	35.68	30.13	46.84
5	2.118	2.0794	29.97	30.53	35.66	30.12	46.81
6	2.090	2.0548	31.19	31.72	35.56	30.36	47.11
7	2.561	2.5459	30.48	30.67	35.60	30.08	46.71
8	1.843	1.8121	30.56	31.09	35.67	spattered	
9	1.946	1.9747	30.17	30.62	35.64	30.32	46.96
10*	2.255	2.2438	30.46	30.61	35.75	30.14	46.91
Average			30.40	30.77	35.66	30.16	46.84

* Collaborative samples 4 and 10 were analysed by the same analyst at different times.

The samples in the glass-topped jars were submitted to the collaborators to determine whether fat which had become separated could be uniformly incorporated by mixing before analysis.

The results obtained by collaborators on samples in the glass-topped jars are given in Table 2. The results under a given sample number were obtained by the same collaborator reporting under the corresponding number in Table 1. The fat on the dry basis is calculated from the average fat and average moisture reported.

TABLE 2

Collaborative results on jar samples

SAMPLE NO.	MOISTURE	FAT	FAT	FAT
		INDIVIDUAL RESULTS	AVERAGE	DRY BASIS
	per cent	per cent	per cent	per cent
1	36.48	29.96	30.09	47.34
	36.49	30.25		
	36.38	30.06		
2	36.43	29.82	29.71	46.71
	36.36	29.76		
		29.54		
3	36.37	30.42	30.35	47.68
	36.27	30.29		
4	35.73	30.29	30.29	47.13
5	36.51	29.50	29.53	46.56
	36.63	29.56		
6	36.15	31.20	31.11	48.75
	36.24	31.03		
	36.14			
7	36.34	30.37	30.35	47.65
	36.26	30.33		
8	35.96	30.43	30.47	47.61
	36.06	30.48		
	35.98	80.50		
9	36.07	(a) 30.44	(a) 30.52	(a) 47.73
	36.06	30.60		
	36.01			
		(b) 30.47	(b) 30.55	(b) 47.77
		30.63		
10	35.77	30.02	30.05	46.77
	35.68	30.09		
11	36.39	30.10	30.35	47.75
	36.41	30.32		
	36.44	30.60		
	36.46	30.39		

[a] Acid digestion performed with the use of sand.
[b] Acid digestion performed without the use of sand.

DISCUSSION

The average fat content of the control samples analyzed in this laboratory immediately after preparation of samples is 30.16 per cent on the original basis, and it varies from 29.97 to 30.32 per cent. The average fat

content of the samples submitted to collaborators in the test-tube beakers is 30.40 per cent. These samples provided a severe test of the manipulative details of the method. The results are quite satisfactory, as only one result (31.72 per cent) is markedly out of line. All collaborators reported separation of fat.

The collaborators' average on the larger samples sent in the glass-topped jars, which required remixing by the analyst in order to obtain a representative sample, is 30.28 per cent. This figure agrees remarkably well with the average original sampling, 30.16 per cent, and with the average of the test-tube beaker samples, 30.40 per cent. Again one figure only, 31.11 per cent, is not in accordance. This result on Sample 6 was obtained by the collaborator that reported high on the other sample.

It is unnecessary to quote all the collaborators' comments as the condition of the samples may be judged by those of one collaborator: " * * * there was considerable fat lying upon the rubber outside the rim of the jar, between that rim and the inner part of the rim of the lid. It is hard to conceive of a cheese sample being in worse condition for analysis."

CONCLUSIONS

The Schmid-Bondzynski method gives concordant results in the hands of different analysts.

A cheese sample in which the fat has become separated before analysis may be remixed and analyzed, if necessary, and the result obtained will be as satisfactory as if the fat had not separated. The recognition of the method as official is warranted.

The official method for moisture in cheese[1] also gives concordant results in the hands of different analysts.

ACKNOWLEDGMENT

DOLOMITE AS A FERTILIZER SUPPLEMENT—ITS BEHAVIOR AND CONTROL[2]

By W. H. MacIntire (University of Tennessee, Knoxville, Tenn.)

During the last few years it has been conclusively demonstrated that there is often need for supplements of magnesia in the so-called "com-

[1] *Methods of Analysis, A.O.A.C.,* 1930, 238.
[2] Presented at the annual meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., November, 1932.

plete" fertilizers. This finding is not restricted to soils within a limited area. The present discussion, however, is, confined to humid regions where acid soils predominate and where fertilizers are most extensively used.

A lack of available magnesia in the soil is definitely registered by the plant through characteristic leaf symptoms. Garner[1] and associates demonstrated that "sand drown" in tobacco is directly due to an insufficiency of magnesium. Murwin[2] likewise demonstrated "the necessity for including magnesia in the fertilizer mixture" for tobacco. Jones[3] showed that the marked decline in yield of corn at the Massachusetts Station, is attributable to depletion of magnesia as a cumulative effect. Chucka[4] showed that in Maine potatoes give a marked response to additions of magnesium. Cooper,[5] of South Carolina, found that a definite "sand drown" in cotton and other crops is corrected by additions of dolomite.

The deficiency of magnesia may occur in a fertilized acid soil and also in a soil that has been limed, but the causes for the same effect under the two conditions are different. In one case the supplies of magnesia are depleted, in the other they are made less soluble. In time, the use of acid phosphate—particularly on light, unlimed soils—will materially reduce the stores of exchangeable magnesium, the amounts of which are as a rule decidedly less than those of calcium, although total magnesium generally exceeds total calcium. That is, with an *acid* soil, the calcium sulfate content of the superphosphate causes a replacement and an increased outgo of exchangeable magnesium and the same is true of potassic salts. On the other hand, the addition of high-calcic liming materials exerts a "protective effect" upon the native supplies of magnesium, due to the fact that the alkaline calcium materials repress the hydrolysis of the magnesic complexes. This has been demonstrated by the lysimeter results of the Tennessee Station.[6] Hence, a deficiency of available magnesium is accentuated by the use of high-calcic liming materials. This finding fits in with the observations of Moss[7] as to the use of calcareous materials for tobacco and the findings of Jones[8] that the addition of high-calcic limestone did not correct a magnesia deficiency for corn.

The need for supplements of magnesia has been recognized and recommended by the Southeastern Tobacco Research Committee.[9] The magnesia additions may be supplied separately, but the present discussion is limited to the inclusion of magnesia compounds as components of mixed goods. Supplements of magnesia may be supplied from two sources, the

[1] *J. Agr. Research*, 23, 27 (1923); Rhode Island Agr. Expt. Sta. Bull. 186 (1921); North Carolina State Dept. Agr. June Bull. (1927); U. S. Dept. Agr. Tech. Bull. 12 (1927); *J. Am. Soc. Agron.*, 21, 142 (1929); 24, 707 (1932).
[2] Connecticut Agr. Expt. Sta. Bull. 299, pp. 197, 198 (1929).
[3] *J. Agr. Research*, 39, 873 (1929).
[4] *J. Am. Soc. Agron.*, 23, 1052 (1931).
[5] South Carolina Expt. Sta. Ann. Rpts. 36 and 38 (1931).
[6] *J. Am. Soc. Agron.*, 18, 482 (1926).
[7] North Carolina State Dept. Agr. June Bull. (1927).
[8] *Loc. cit.*
[9] South Carolina Expt. Sta. Am. Rpts. 36 and 38 (1931).

quantities supplied by cottonseed meal and barnyard manure being disregarded. It was stated by Cooper that the extensive use of cottonseed meal in the past probably prevented the earlier appearance of the prevalent symptoms of magnesia deficiency in cotton grown in the Sand Hill section of South Carolina. To effect immediate results, readily soluble salts may be furnished through the magnesium sulfate content of low-grade potash salts. Lysimeter findings at the Tennessee Station have shown, however, that magnesium sulfate passes out of the soil very rapidly, much more so than calcium sulfate.[1] In sandy soils of the Coastal Plains, this rapid outgo is even more pronounced, and with heavy rainfall, annual or even more frequent additions of soluble magnesium sulfate might be required. Furthermore, the MgO content of the ordinary magnesium sulfate is only 16.4 per cent; hence, it is not always feasible to supply the recommended 2 per cent minimum requirement from this source.

The alternative practice of the inclusion of dolomite as a supplement, and in lieu of an inert filler, has several advantages. The excess of free phosphoric acid is neutralized and the mix shows a good mechanical condition. Dolomite also insures a sustained supply of magnesium during the persistence of the carbonates and also subsequent to fixation of the added bases, through the biological generation of bicarbonates, nitrates, and sulfates, especially the latter. In particular, the increased use of ammonium sulfate in "complete" fertilizers necessitates the parallel use of natural carbonate fillers, or supplements. In this way the cumulative, detrimental acidity that follows the continued use of ammonium salts is prevented and instead a gradual accumulation of exchangeable bases is obtained. It has been shown by MacIntire and Sanders[2] that dolomite may be mixed with several ammonium salts without loss of ammonia, and Parker[3] has forcefully pointed out the advantages and economics that would result to the fertilizer manufacturer if dolomite or limestone were included in the mix.

In a consideration of the inclusion of dolomitic limestone as a component of mixed fertilizers, several antiquated prejudices need to be overcome. When practice was determined, or at least influenced, by these prejudices, little was known of the differential behavior of high-calcic limestone and dolomite, either in fertilizer mixes or after incorporation with the soil. In the first place, dolomite can be used in any proportion and fineness, whereas high-calcic limestone cannot be used *ad libitum* in commercial mixes, because of the monetary penalty that will follow when the present official methods are used. This factor does not apply, however, in the case of home mixes that are intended for immediate use.

The official methods for P_2O_5 were intended and perfected for acid-reactive superphosphate, or its mixes, and it was never contemplated that

[1] *Soil Sci.*, 16, 1, 1b9 (1923).
    J. Am. Soc. Agron., 20, 764 (1928).
[3] *Ibid.*, 24, 707 (1932).

they would be used to analyze materials that contain the natural carbonates of the alkaline earths. Under some conditions, such as humidity, large proportions of high-calcic limestone, and extended aging, a tri-phosphate of calcium may be formed in superphosphate-limestone mixtures. It has been shown, however,[1] that even then the larger part of the registered occurrence of insoluble P_2O_5 is brought about during the analytical process. Furthermore, the solvent power of the ammonium citrate solution is considerably decreased by its digestion-reaction with the undecomposed fraction of the included high-calcic limestone. Hence, the residue from the citrate digestion will register more "insoluble" P_2O_5 than was actually present in the analytical charge. This effect is lessened, although not entirely eliminated, by the use of the 1-gram charge. The restriction upon the use of high-calcic limestone supplements is therefore primarily a question of commercial evaluation, based on control analysis. There is considerable evidence, however, that there is no justification in giving to precipitated tricalcium phosphate a fertility value materially less than that assigned to the mono- and di-forms.

The numerous publications of Loew and his associates in this country, in Germany, and in Japan were responsible for unwarranted fear concerning the toxic effects that may be produced by magnesium salts, added or engendered, in the soil. When pure carbonates of calcium and magnesium are used in sand cultures, or when soluble salts of calcium and magnesium are used in water cultures, plant-response results are not the same as those that are obtained when buffered soils are judiciously limed with natural carbonates. Furthermore, the mechanical mixtures of oxides or carbonates of calcium or magnesium do not function in the same way as do the natural carbonates found in dolomitic limestones. But, based on erroneous deductions from the earlier experiments, there existed strong prejudices against the use of dolomitic limestone. It is true that with injudicious liming, toxicity may be induced by excessive quantities of resultant solute magnesia with attendant paucity of calcium, just as, conversely, toxicity may be caused by an excess of calcium and a paucity of magnesium, both of these conditions being accompanied by a repressive effect upon solubility of potassium and other elements in the soil.[2] An excess of either form of limestone is also favorable to the development of the root rots. It is nevertheless true, as will be discussed later, that an economic and judicious use of dolomite will not produce a toxic effect. Agronomic studies at the Tennessee Station have demonstrated that the two types of limestone give comparable results for crops in general, with frequent evidence of superiority of the dolomite, especially for specific crops. Similar findings have been published by the New Jersey,[3] Indiana,[4] and Rhode Island[5] Stations.

[1] *Ind. Eng. Chem.*, 24, 933 (1932).
[2] *J. Agr. Sci.*, 20, 499 (1930).
[3] Bull. 430 (1926), 498 (1930), *Soil Sci.*, 15, 307 (1923).
[4] J. Agr. Research, 18, 119 (1919), Agr. Expt. Sta. Bull. 329 (1929).
[5] *Soil Sci.*, 18, 169 (1924), Agr. Expt. Sta. Bull. 186 (1921).

The characteristic activities of the two forms of limestone in fertilizer mixes and also in the body of the soil will now be considered, and particular attention given to their influence on magnesium availability to the plants. It is well known that dolomites are less soluble than limestone, but this difference in solubility is minimized when dolomite is finely ground. When dolomite was first proposed as a filler, or conditioner, without emphasis on the supplemental value of its magnesium content, it was assumed that there would be no extensive reactivity between the superphosphate and the added dolomite. It developed, however, that considerable reaction does take place, though not to the extent found for limestone-superphosphate mixes. Nevertheless, in large bulk mixes no loss in weight is noted.

Two recent publications of the Tennessee Station[1] have established several facts relative to the activities that transpire in dolomite-superphosphate mixes. In both of these studies, the evolution of CO_2 was taken as the measure of the speed of the reactions. In one instance the CO_2 evolved from dry mixes was aspirated continuously and determined periodically for 6 months, whereas the P_2O_5 transitions were measured by the official methods, 75 days and 6 months after mixing. At the end of those periods no decrease in "available" P_2O_5 was found in the aged dolomite mixes. The P_2O_5 equivalent of the evolved CO_2 is therefore a measure of the formation of diphosphates in superphosphate-dolomite mixes. Furthermore, contrary to expectancy, the very fine material, though initially more reactive, gave a smaller ultimate reaction, as measured by evolved CO_2. This was attributed to the spread of moisture over the greater surface offered by the finer particles. It was also found that dolomite produces a nugatory change in the solvent capacity of neutral ammonium citrate, a point that was also brought out by Jacob, et al.[2] The loss of weight that resulted from evolutions of CO_2 from the dry mixes was compensated for by gain in water of crystallization through the formation of a trihydrate, di-magnesium phosphate. Formation of the di-phosphate of magnesium is responsible for an enhanced and accelerated drying-out effect that reduces the effective moisture. Furthermore, there occurs no reaction between dolomite and either of the engendered di-calcic or di-magnesic phosphates. In the other instance, the reactions were studied in aqueous suspensions and wet mixes, the periodic determinations of evolved CO_2 being supplemented by determinations of the solute Ca, Mg and P_2O_5. The reactions that transpire in aqueous suspensions[1] differ materially from those that transpire in moist mixes and dry bulk mixtures. On the basis of the results obtained in two studies it was concluded that no tri-phosphates are formed in dry dolomite-superphosphate mixes, irrespective of the degrees of fineness of the dolomite. The dolomite additions also produced considerable quantities of di-phosphate of magne-

[1] *Ind. Eng. Chem.*, 24, 933 (1932).
[2] *This Journal*. 15, 159 (1932).

sium, which is much more available than the corresponding calcium phosphate.

There is the further point of the activities of that undisintegrated fraction of the dolomite supplement that is incorporated in the soil along with the fertilizer. Here, again, dolomite differs decidedly from limestone. As previously stated, high-calcic materials will diminish the solubility of native magnesium compounds, whether the calcium of the added caustic or carbonate forms continues unfixed as carbonate or becomes fixed in the absorption complexes. In both cases the hydrolysis of the native magnesium compound is decreased. Again, although the total supplies of magnesium in the soil almost always exceed those of calcium, the amount of replaceable magnesium is always decidedly less than the amount of replaceable calcium. Hence, in Tennessee soils, the ratio of Ca to Mg in the percolates from an unlimed acid soil will usually be 2:1 or 2.5:1. Additions of dolomite increase the amount of magnesium, but not necessarily the amount of calcium that is found in the natural percolates. The nature of the soil determines the manner in which this increase is brought about. In "heavy" soils of high-fixation capacity, additions of finely ground dolomite are soon "fixed" and held as non-carbonate forms by the soil complex. The "fixed" magnesium undergoes hydrolysis more readily than does the fixed calcium. Hence, in the heavier types of soil, the free soil water is considerably enriched with magnesium. The increase in calcium, however, may be small, or even negative. If the availability of the native supplies of calcium is not materially changed, coincident with the enhancement of soluble magnesium that results from the dolomite addition, the ultimate effect is to give a ratio approaching 1:1 for the Ca:Mg content of the free soil water. As measured by outgo of calcium and magnesium, the ratio found in percolates from dolomite additions in heavy soils seldom varies appreciably from that value.

It should be stressed that the use of dolomite for tobacco and cotton on light sandy soils is prescribed primarily as a source of magnesium and to neutralize acidity resultant from added ammoniates, rather than as an amendment for existent soil acidity. In these soils of high quartz and low colloid content, the disintegration of the dolomite is more a matter of weathering, or direct solvent action. Under such conditions, the calcite that occurs in the interstices between the true dolomite crystals undergoes preferential solution, so that the early solutions of added dolomite are apt to be somewhat richer in calcium than in magnesium. In time, however, the ratio of solute calcium-magnesium reaches the true dolomite ratio of 1:1, so that in the free water of sandy soils there can never be an excess of magnesium over calcium. In unpublished data from the Connecticut Station, Morgan showed that separate additions of 100 to 600 pounds of high-magnesic lime materially increased the magnesium content and decreased the calcium content in the ash of tobacco plants, which confirms

previous findings by Anderson and Swanback,[1] who used hydrated lime that was presumably high-magnesic.

Here, it is well to point out that the terms "dolomite" and "dolomitic" are often used incorrectly in referring to magnesian, or high-magnesic, limestone. By definition, a dolomite is a rock that contains calcium and magnesium carbonates in the molar ratio of 1:1. Such rocks are found in Georgia and Tennessee, but the usual "dolomite" is one in which the occurrence of calcium exceeds that of magnesium. In ordinary parlance, however, any rock that contains a magnesium carbonate content of 30 per cent is designated as a dolomite.

The disintegration of "dolomitic" limestones of varying ratios and purity in carbonated water has been studied by MacIntire and Shaw.[2] The reactions of carbonated water and of phosphoric acid upon dolomites show an interesting parallel. From results obtained in aqueous suspensions with mono-calcium phosphate, it appears that the excess of $CaCO_3$ in a dolomite rock acts just as would an equivalent amount of calcite added to a true dolomite. It has been also pointed out by MacIntire and Shaw[3] that the proportions of di-phosphate of calcium and magnesium in superphosphate mixtures with dolomite will depend upon the nature of the rock, whether it be a high-magnesic, a "near" dolomite, or a true dolomite.

The effect of pre-mixing dolomite with superphosphate should be considered from another angle. When water-soluble P_2O_5 is incorporated with a soil there is apt to follow a quick fixation of phosphate with iron and aluminum, where sufficient quantities of alkaline earths are absent. In pre-mixed phosphates, however, the amount of water-soluble P_2O_5 is materially lessened by the formation of equivalent quantities of the di-phosphates. The fixation of these forms in the soil is much less rapid, and there exists considerable evidence that di-phosphates give results superior to those obtained from mono-phosphate on some soils. Furthermore, certain lysimeter evidence is already at hand to indicate that the mobility of the magnesium phosphates in the free soil water exceeds that found for the calcium phosphates. This may be of practical importance in crops such as wheat, where the content of the grain is high in both phosphorus and magnesium, and low in calcium.

If the use of dolomite as a supplement is acknowledged and placed under control, the practice will increase coincidentally with an understanding of its value and the established fact that neither the material nor its reaction products will result in penalties by the control chemists. In one state, however, the inclusion of any form of limestone has been prohibited and in no state is any commercial value assigned to the calcium and magnesium carried by it, although the manufacturer is permitted to label the

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[1] Connecticut Agr. Expt. Sta. Bull. 299, pp. 197, 198 (1929).
[2] *J. Am. Soc. Agron.*, 22, 14 (1930).
[3] *Loc. cit.*

container to show the use of a definite quantity of dolomite, or of lime-stone. If dolomite supplements are used, statement to that effect not only should be permitted, but required, and control should be established. This point is now under consideration in one state, and the question has been raised as to methods to be used in control work.

The farmer certainly derives both immediate and cumulative benefits from dolomite supplements which insure him against those physiological disturbances and decreased yields that are caused by magnesium defi-ciency. He should be informed as to the inclusion of supplements and pro-tected against unwarranted claims.

It should be borne in mind also that dolomite may be used advantage-ously with both the so-called low-grade goods and the concentrates. It is certain that manufacturers who use dolomite supplements supply a prod-uct of superior physical properties and one that will not disintegrate the containers. Certainly, if fillers are to be used, dolomite is preferable to inert materials, and the progressive manufacturer is entitled to considera-tion. Probably the principal deterrent is the fact that dolomite is more expensive than inert fillers and no credit for its use is allowed in evaluating the finished product. This economic factor cannot be ignored.

This brings us to the point that is of direct concern, and a responsibility, of this Association—the need of methods to determine the amounts and forms of calcium and magnesium supplements. It would not be difficult to prescribe the analytical work necessary for magnesium alone, when this supplement is incorporated as a soluble salt. The method of expressing the results would be the only point to be settled. The problem of calcium and magnesium supplements, in relation to limestone or dolomite, is, however, not so simple. It might develop that the use of limestone or dolomite would be permitted and declaration required, without stipula-tion as to amounts. If a qualitative determination only were specified to differentiate between limestone and dolomite supplements, the problem would be simple. A large fraction of an added limestone is to be found in the insoluble residue from the aqueous extraction and the subsequent ammonium-citrate digestion. If the particles were not too small, the resistance of these carbonate residues to weak acids, such as phosphoric and acetic, could be used to identify dolomite. The resistance of dolo-mite to weak acids is materially lessened, however, when the dolomite is in a fine state of division. In this case the specific ferric chloride and molybdenum tests could be utilized.

It is almost certain, however, that the additions of calcium and mag-nesium supplements, especially the latter, will be considered on the quan-titative basis, and here a number of difficulties appear. Immediately arises the question whether claim should be permitted solely for the amounts of residual carbonates, or for the full additions which are partly disintegrated by reaction with free phosphoric acid and mono-calcium phosphate. In

other words, is credit to be given for the amounts of calcium and magnesium utilized in the neutralization of the free phosphoric acid and for one-half of the calcium and magnesium that is carried by di-phosphates formed by the reactions between mono-calcium phosphate and dolomite? Superphosphate contains variable amounts of free phosphoric acid and they usually carry some di-phosphate, hence, the amount of calcium present in this form in dolomite mixes cannot be ascribed solely to the added dolomite. In view of the low content of magnesium usually found in phosphate rock, however, it would seem reasonable to ascribe to dolomite additions the amount of magnesium present as a di-phosphate in superphosphate-dolomite mixes. Another possibility would be to determine total calcium and magnesium and to give credit for the amounts of the two elements not accounted for jointly by calcium sulfate, mono- and tri-phosphates. This would involve considerable analytical work and computation, and here would arise the question as to mode of expression. Should it be as calcium and magnesium oxides, or as the two carbonates, or as $CaCO_3$-equivalent?

It seems equitable that the manufacturer should be given credit for the full amount of dolomite incorporated with the mix. If it were feasible to compare the initial composition of the superphosphate with the composition of the superphosphate-dolomite mix, an accurate check could be had. Nevertheless, in control it would be difficult to verify claims for such incorporation, if these were made for the full incorporations of both calcium and magnesium. But if claims for use of dolomite were confined solely to the magnesium supplements, it would not be difficult to verify such claims.

Matters would be simplified greatly if the claim for calcium and magnesium supplements were based on the amounts of dolomite present as such in mixed goods at the time of sale. That is—disregarding the factor of the neutralization of the free phosphoric acid content of the superphosphate—if no claim were made for the amounts of calcium and magnesium that represent a difference between the amounts of calcium carried by mono-calcium phosphate in the superphosphate and the amounts of calcium and magnesium required to convert a part of the original mono-calcium phosphate to di-phosphate, control methods could be worked out without great difficulty. By reference to the curves given by MacIntire and Shuey,[1] it will be seen that after the initial period of accelerated activity due to the free phosphoric acid and maximum effective moisture, the disintegration of dolomite decreases coincidentally with drying-out effects that transform much of the effective moisture into water of crystallization required by the di-phosphate, particularly di-magnesium phosphate. Hence, in a well-cured superphosphate-dolomite mix, no considerable change in the dolomite content would be expected during the period

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[1] *Loc. cit.*

elapsing between samplings at the factory and those made for control analyses. Since the water-extraction and the citrate-digestion would remove all magnesium phosphate, the analysis of the residue to determine the amounts of P_2O_5, calcium, and magnesium would establish the residual quantity of dolomite and its proportions of calcium and magnesium. To determine further that the dolomite carried by the leached and digested residue is truly a measure of the amount present in the analytical charge, that is, that no decrease in dolomite content is caused by the analytical procedure, the CO_2 content of the residue would be compared with the CO_2 content of the original analytical charge.

Even though the development of methods for control should prove to be more difficult than anticipated, it is certain that these could be perfected. It is evident that station workers and commercial interests are being converted rapidly to the advantages accruing to the use of dolomite as a source of magnesium supplements. It follows that, in time, state laws will prescribe that the use of dolomite shall be permitted and its presence stipulated; and further that claims, especially for added magnesia, shall be subject to verification by the official methods. It also follows that this Association will be expected to perfect workable methods for the control of the use of supplements of limestone, and of dolomite in particular.

AVAILABILITY OF IRON, ALUMINUM, AND OTHER PHOSPHATES[1]

By R. P. BARTHOLOMEW and K. D. JACOB[2]

Interest in experiments relating to the fertilizer value of insoluble phosphates has been stimulated by the production of ammoniated superphosphates, and, as a result, several valuable contributions to the knowledge of the phosphorus nutrition of plants have been made recently. The studies have been conducted for the most part, however, with various calcium phosphates. In addition to the calcium compounds produced in the manufacture of straight and ammoniated superphosphates, varying amounts of iron and aluminum phosphates are formed also.

The availability of iron and aluminum phosphates as sources of phosphorus for plant growth in quartz sand has been investigated by Truog[3] and others, who reported a rather high availability for the phosphorus in precipitated iron and aluminum phosphates. The availability of ferric phosphate was attributed to a hydrolysis reaction, which liberated phosphoric acid and caused the undissolved phosphate residue to become more basic and less available to plants. It was assumed that aluminum

phosphate behaves in a similar manner. Brioux,[1] from the results of experiments conducted in jars with soils fertilized alike with the exception of the phosphorus which was supplied as iron, aluminum and tricalcium phosphates, respectively, reported that aluminum phosphate produced more growth than tricalcium phosphate with flax, spurry, buckwheat and yellow clover. Of the plants used in the experiments, barley was the only one that made better growth with ferric phosphate than with either tricalcium or aluminum phosphate. Austin[2] concluded from the results of his experiments, that the presence of aluminum in acid soils does not make soluble phosphates insoluble if other compounds are present which may react with both aluminum and phosphoric acid.

In view of the fact that results of recent studies[3] concerning the availability of some phosphatic compounds of calcium, formerly regarded as unavailable, have caused certain changes to be made in the official method of determining available P_2O_5, it seemed desirable to make further investigations concerning the availability of iron, aluminum and other phosphates when used as fertilizers in acid soils. Experiments were undertaken, therefore, to determine the relative fertilizer efficiency of such phosphates as compared with their availability determined by the official method of the A. O. A. C.

PREPARATION AND COMPOSITION OF PHOSPHATES

Aluminum Phosphates.—Aluminum phosphate was prepared by slowly adding, with constant stirring, a 0.2 *M* solution of triammonium phosphate (prepared by adding the proper quantity of ammonium hydroxide to a solution of diammonium phosphate) to a 0.1 *M* solution of aluminum sulfate $(Al_2(SO_4)_3 \cdot 18H_2O)$. After being washed with water until the filtrate gave only a slight test for ammonia, the precipitate was dried at approximately 50° to 60° C. The product contained P_2O_5 36.49, Al_2O_3 26.72, Fe_2O_3 0.025, and NH_3 0.13 per cent, the ratio of alumina to phosphoric acid being very close to the theoretical ratio for $AlPO_4$

A portion of this material was ignited in 25-gram quantities for 2 hours at 1000° C. in an electric muffle furnace. The product contained 54.14 per cent P_2O_5.

The natural aluminum phosphate used in this investigation came from a deposit in the Connetable Islands, which are located 13 miles off the coast of French Guiana in the approximate position latitude 4°54′ North and longitude 51°57′ West. The material contained P_2O_5 42.34, Al_2O_3 28.57, and Fe_2O_3 3.42 per cent. No calcium was present. The ignited product, which was prepared by heating 50-gram portions of the 100-mesh powder for 2 hours at 800° C. in an electric muffle furnace, contained 55.37 per cent P_2O_5.

[1] *Ann. sci. agron.*, **40**, 185 (1923).
[2] *Soil Sci.*, **24**, 263 (1927).
[3] Ross, Jacob and Beeson. *This Journal*, **15**, 227 (1932).

Ferric Phosphates.—Ferric phosphate was prepared by mixing 0.1 M solutions of triammonium phosphate and ferric ammonium sulfate ($Fe_2(SO_4)_3 \cdot (NH_4)_2SO_4 \cdot 24H_2O$), the procedure being the same as that used for the preparation of aluminum phosphate. The product contained P_2O_5 32.78, Fe_2O_3 41.75, Al_2O_3 0.07, and NH_3 0.19 per cent. In proportion to the phosphoric acid, it contained an excess of approximately 13 per cent of iron above that theoretically required for $FePO_4$. The ignited material, which was prepared by heating 25-gram quantities for 2 hours at 800°C. in an electric muffle furnace, contained 43.71 per cent P_2O_5.

Calcium Metaphosphate.—This material was prepared by heating acid-free monocalcium phosphate to constant weight at 600° to 650°C., the temperature being increased gradually in order to avoid fusion. The product was obtained in the form of very hard fragments, which were practically insoluble in aqua regia but dissolved in hot concentrated sulfuric acid. It contained 71.04 per cent P_2O_5 and 28.42 per cent CaO. This particular material was insoluble in neutral ammonium citrate solution, but it should be borne in mind that the metaphosphates exist in several forms which differ considerably in their physical and chemical properties. Attempts to prepare pure calcium metaphosphate by double decomposition were not successful.

Calcium Pyrophosphate.—Calcium pyrophosphate was prepared by heating pure dicalcium phosphate to constant weight at 800°C. The product contained 55.56 per cent P_2O_5 and 44.38 per cent CaO. This compound was also prepared by mixing a 7 per cent solution of Merck's sodium pyrophosphate ($Na_4P_2O_7 \cdot 10H_2O$) with an excess of a 20 per cent solution of calcium chloride. The product obtained upon washing the precipitate free from chlorides and drying at 50° to 60°C. contained 45.71 per cent P_2O_5 and 35.97 per cent CaO, the ratio of lime to phosphoric acid being very close to the theoretical for $Ca_2P_2O_7$.

Potassium Metaphosphate.—This material was prepared by heating pure monopotassium phosphate in a platinum dish to constant weight at 810° to 820°C. Since this compound was prepared in the same way that Beans and Kiehl[1] prepared sodium monometaphosphate, it is probable that the salt thus obtained is potassium monometaphosphate (KPO_3). The product analyzed 60.22 per cent P_2O_5, 2.91 per cent water-soluble P_2O_5, as determined by the official method,[2] and 40.21 per cent total K_2O.

Potassium Pyrophosphate.—This compound, which was prepared by heating pure dipotassium phosphate in platinum to constant weight at 1000°C., contained 42.80 per cent total P_2O_5 and 56.95 per cent K_2O. It was completely soluble in cold water.

Magnesium Phosphates.—The dimagnesium phosphate, which was

[1] *J. Am. Chem. Soc.*, **49**, 1878 (1927).
[2] *Methods of Analysis, A.O.A.C.*, 1930, 16.

Kahlbaum's C. P. material, contained 40.31 per cent P_2O_5 and 23.31 per cent MgO.

Trimagnesium phosphate was prepared by slowly adding a solution of trisodium phosphate to a solution containing an excess of magnesium sulfate. The precipitate was washed with cold water until the filtrate gave no test for sulfates, and was dried at a temperature of approximately 75°C. The product analyzed 39.77 per cent P_2O_5 and 34.06 per cent MgO.

Magnesium ammonium phosphate was prepared by precipitating an aqueous solution of pure phosphoric acid with ammoniacal magnesium chloride solution. The precipitate, after drying for several weeks over concentrated sulfuric acid, contained P_2O_5 29.98, MgO 16.76, and NH_3 7.16 per cent.

AVAILABILITY OF PHOSPHATES AS DETERMINED BY THE AMMONIUM CITRATE AND CITRIC ACID METHODS

The samples were analyzed for citrate-soluble phosphoric acid by the recently adopted modification of the official method, which involves the digestion of 1-gram samples with 100 cc. of neutral ammonium citrate solution for 1 hour at 65°C.[1] For purposes of comparison, the samples were also analyzed for phosphoric acid soluble in 2 per cent citric acid according to the official method for the evaluation of phosphoric acid in basic slag.[2]

The results, which are given in Table 1, indicate a high plant food value for the phosphoric acid in the magnesium phosphates, synthetic aluminum phosphate, and unignited synthetic ferric phosphate. Although ignition has no effect on the citrate solubility of synthetic aluminum phosphate, it decreases the solubility of synthetic ferric phosphate to a marked extent. On the other hand, ignition has a very marked effect in increasing the citrate solubility of natural hydrated aluminum phosphate, which is in line with the results obtained by Morse[3] in experiments on hydrated aluminum phosphate from Redonda Island. The iron and aluminum phosphates are much more soluble in neutral ammonium citrate than in 2 per cent citric acid solution, as shown previously by Jacob, Rader, and Ross.[4]

The citrate extracts of potassium metaphosphate were exceedingly difficult to filter and wash, approximately 20 hours being required for the completion of these operations. During this time, considerable hydration of the metaphosphate to the pyro- and orthophosphates undoubtedly occurred.

[1] *This Journal*, **15**, **43**, **65** (1932).
[2] *Methods of Analysis*, A.O.A.C., 1930, p. 27.
[3] *J. Am. Chem. Soc.*, **25**, 280 (1903).
[4] *This Journal*, **15**, 146 (1932).

TABLE 1

*Availability of phosphates as determined by the ammonium
citrate and citric acid methods*

MATERIAL	TOTAL P₂O₅	AVAILABLE P₂O₅				FINENESS OF MATERIAL
		AMMONIUM CITRATE METHOD		CITRIC ACID METHOD		
		OF SAMPLE	OF TOTAL P₂O₅	OF SAMPLE	OF TOTAL P₂O₅	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*mesh*
Calcium Metaphosphate.....	71.04	0.00	0.0	0.63	0.9	200
Calcium Pyrophosphate, prepared by heating dicalcium phosphate...	55.56	2.58	4.6	2.36	4.2	200
Calcium Pyrophosphate, prepared by double decomposition...................	45.71	23.88	52.2	36.19	79.2	80
Potassium Metaphosphate...	60.22	58.79	97.6	34.67	57.6	80
Potassium Pyrophosphate...	42.80	42.80	100.0	42.80	100.0	80
Dimagnesium Phosphate....	40.31	40.31	100.0	40.31	100.0	80
Trimagnesium Phosphate....	39.77	39.77	100.0	39.77	100.0	80
Magnesium Ammonium Phosphate..................	29.98	29.98	100.0	29.98	100.0	80
Ferric Phosphate, unignited..	32.78	32.78	100.0	3.85	11.7	80
Ferric Phosphate, ignited....	43.71	13.34	30.5	1.65	3.8	80
Aluminum Phosphate, synthetic, unignited............	36.49	36.49	100.0	13.60	37.3	80
Aluminum Phosphate, synthetic, ignited............	54.14	54.14	100.0	7.85	14.5	80
Aluminum Phosphate, natural, unignited..........	42.34	3.78	8.9	0.25	0.6	100
Aluminum Phosphate, natural, ignited	55.37	42.23	76.3	1.57	2.8	100
Monocalcium Phosphate, C. P.	56.10	56.10	100.0	56.10	100.0	40
Superphosphate*...........	19.32	19.03	98.5	17.80	92.1	40

* Commercial material made from Florida pebble phosphate rock.

EFFECT OF IGNITION ON THE CITRATE SOLUBILITY OF IRON AND ALUMINUM PHOSPHATES

In carrying out the experiments relating to the effect of ignition at various temperatures on the citrate solubility of ferric and aluminum phosphates, 1-gram samples were heated for the desired length of time, and the entire residue from the ignition was used directly for the determination of citrate-soluble phosphoric acid, the results being expressed on the basis of the original unignited material. In order to obtain clear filtrates, the citrate extracts were filtered through short Pasteur-Chamberland tubes.

The results (Table 2) show that the citrate solubility of the phosphoric

TABLE 2

Effect of ignition on the citrate solubility of natural hydrated aluminum phosphate and synthetic ferric phosphate

TEMPERATURE OF IGNITION	IGNITION LOSS	CITRATE-SOLUBLE P_2O_5
°C.	per cent	per cent
Natural Aluminum Phosphate[a]		
Unignited	—	3.78[b]
105°	22.54	26.85
200	22.33	25.24
400	22.76	25.90
600	23.28	27.26
800	23.60	25.84
Synthetic Ferric Phosphate		
Unignited	—	32.78[d]
105[e]	12.75	32.78
300	22.85	32.78
500	23.58	32.78
700	24.33	7.92
900	25.43	1.97

[a] From the Connetable Islands.
[b] Total $P_2O_5 = 42.34$ per cent.
[c] Heated to constant weight. Other samples heated for 2 hours.
[d] Total $P_2O_5 = 32.78$ per cent.
[e] Heated to constant weight. Other samples heated for 3 hours.

acid in natural hydrated aluminum phosphate from the Connetable Islands is increased from 9 to 63 per cent by heating the material to constant weight at 105°C., the loss in weight at this temperature amounting to 22.54 per cent. Although the results are somewhat erratic, no further significant change in citrate solubility is obtained by heating this material at higher temperatures, at least up to 800°C. Morse[1] reported, however, that with aluminum phosphate from Redonda Island the maximum solubility in neutral ammonium citrate solution was obtained on material heated at 560°C., whereas the solubility showed a marked decrease from the maximum in samples heated at 830°C. Heating at temperatures up to 1100°C. had no effect on the citrate solubility of the sample of synthetic aluminum phosphate.

As shown in Table 2, heating at temperatures as high as 500°C. has no effect on the citrate solubility of the phosphoric acid in synthetic ferric phosphate. The solubility is affected very adversely, however, by heating the material at 700°C., and in samples heated at 900°C. only 6 per cent of the total phosphoric acid is soluble in citrate solution.

The results given in Table 2 show that the natural aluminum phos-

[1] *J. Am. Chem. Soc.*, **25**, 280 (1903).

phate contains 61 per cent of its total phosphoric acid in the citrate-soluble condition when 1-gram samples are ignited at 800°C. and the determination of citrate solubility is made directly on the entire ignition residue. On the other hand, the results given in Table 1 show that the citrate solubility of the P_2O_5 in this material amounts to 76.3 per cent when the determination is made on 1-gram samples of the residue resulting from ignition at 800°C., despite the fact that the analytical sample contained 1.3 times as much total phosphoric acid as in the first case. In other words, the citrate solubility of ignited aluminum phosphate increases with increase in the weight of sample taken for analysis, a fact noted previously by Huston.[1] In this respect, the behavior of ignited aluminum phosphate is directly opposite to that of the unignited material and of the calcium phosphates,[2] the percentage solubility of the total P_2O_5 in these materials decreasing with increase in the weight of the analytical sample.

Ignited synthetic ferric phosphate is similar to ignited aluminum phosphate in that its citrate solubility increases with increase in the weight of sample taken for analysis.

AVAILABILITY OF PHOSPHATES AS DETERMINED BY PLANT GROWTH

The experiments were carried out with a Clarksville silt loam having a pH of 5.7 and known to be responsive to phosphatic fertilizers, the various phosphates being used at the rate of 0.3 gram of total P_2O_5 per jar. Previous experiments have shown that this amount of P_2O_5 is insufficient for maximum growth of plants on this soil. In addition, sodium nitrate and ammonium sulfate sufficient to make a final rate of 150 pounds each per acre and potassium chloride at a final rate of 300 pounds per acre were added in solution and thoroughly mixed in the soil at the time of incorporation of the phosphate material. The equivalent of the amounts of nitrogen and potassium in the phosphate materials was deducted from the solution applications of nitrogen and potassium.

Sudan grass was chosen as the test crop because it is not a strong feeder and is well adapted for greenhouse studies under the climatic conditions existing in Arkansas. In addition, results from collaborative tests reported by Ross, Jacob and Beeson[3] show that it compares favorably with other crops as an indicator of the fertilizer efficiency of phosphate materials in soils having a pH below 6.0. The Sudan grass was seeded March 28, 1932. The soil was watered with distilled water during the entire experiment and was top dressed, after each crop was harvested, with the same amounts of nitrogen and potassium fertilizers as were added

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[1] *Proc. 7th Ann. Convention Assoc. Official Agr. Chem.*, U. S. Dept. Agr., Div. Chem. Bull. **28**, 170 (1890).
[2] Jacob, Beeson, Rader and Ross, *This Journal*, **14**, 263 (1931).
[3] *This Journal*, **15**, 227 (1932).

at the beginning of the experiment. The experiments were made in triplicate and the grass was cut from each jar on May 31, July 25, and September 3, dried in the oven and weighed. The average results for each treatment are given in Table 3.

TABLE 3

Average yields of Sudan grass grown in Clarksville silt loam soil and fertilized with various phosphates.

SOURCE OF PHOSPHORUS	AVERAGE OVEN-DRY WEIGHT OF PLANTS			
	FIRST CUTTING	SECOND CUTTING	THIRD CUTTING	TOTAL
	grams	*grams*	*grams*	*grams*
Calcium Metaphosphate (heat)........ .	32.2	27.3	16.6	76.1
Calcium Pyrophosphate (heat)..	28.4	24.6	20.7	73.7
Calcium Pyrophosphate (double composition).	33.5	26.9	23.1	83.5
Potassium Metaphosphate (heat).	36.6	30.4	22.3	89.3
Potassium Pyrophosphate (heat)... .	34.1	30.6	23.2	87.9
Dimagnesium Phosphate......... ..	37.4	28.9	20.5	86.8
Trimagnesium Phosphate.........	38.9	29.6	22.6	91.1
Magnesium Ammonium Phosphate... .	36.2	32.4	24.0	92.6
Ferric Phosphate............... . .	33.6	27.1	18.9	79.6
Ferric Phosphate, ignited...	34.2	25.7	21.8	81.7
Aluminum Phosphate, synthetic.... . .	39.9	29.9	21.2	91.0
Aluminum Phosphate, synthetic, ignited.... .	37.1	29.9	22.2	89.2
Aluminum Phosphate, natural......	35.4	26.5	22.0	83.9
Aluminum Phosphate, natural, ignited.. .	39.4	27.6	25.8	92.8
Monocalcium Phosphate......	40.0	31.5	20.4	91.9
Superphosphate............	40.1	29.2	20.6	89.9
No phosphorus........	28.8	27.7	21.4	77.9

The differences in yield from the jars that had received monocalcium phosphate and those that had not received any phosphorus serve as indications of the phosphorus needs of the soil. An increase of 38.9 per cent in the first cutting due to the application of monocalcium phosphate, shows that there is a deficiency of available phosphorus in the soil. The results obtained, therefore, should be a reliable index to the fertilizer efficiency of the different materials used in the experiment.

The results from the first cutting show that as much growth was made when the phosphorus was supplied as superphosphate or synthetic aluminum phosphate and almost as much growth from ignited natural aluminum phosphate and trimagnesium phosphate as when the phosphorus came from monocalcium phosphate. On the other hand, magnesium ammonium phosphate, dimagnesium phosphate, ignited synthetic aluminum phosphate, and potassium metaphosphate, which were completely or almost completely soluble in citrate solution, caused only three-fourths as much growth as monocalcium phosphate. Only about 50

per cent as much increase was obtained with potassium pyrophosphate and synthetic ferric phosphate, which contained all their phosphorus in citrate-soluble forms. The failure of these phosphate materials to produce as much growth as monocalcium phosphate can only be attributed to some physiological condition in the plants, since the phosphorus was taken up readily by the plants, as shown later (Table 5).

The phosphoric acid in the unignited synthetic ferric phosphate was all citrate-soluble and yet not quite as much growth was produced with this material as with the ignited synthetic ferric phosphate in which less than one-third of the total phosphorus was citrate-soluble. The natural aluminum phosphate, which contained only 3.78 per cent of citrate-soluble phosphoric acid, produced 62 per cent as much increase in growth as the ignited natural aluminum phosphate, which contained 42.33 per cent of citrate-soluble phosphoric acid. The relative efficiencies of the aluminum phosphates are of particular interest, since these materials seem, as a whole, to be much more available than the ferric phosphates. The significance of this will be discussed later.

The increased yields from the phosphatic fertilizers at the second cutting were small, if any. Slight increases were obtained from all but the calcium meta- and pyrophosphates, iron phosphates and natural aluminum phosphates. The average increased yields in the third cutting were still smaller and occurred in fewer instances.

As regards the total weights of dry matter obtained from the three cuttings, it will be noted that trimagnesium phosphate, magnesium ammonium phosphate, synthetic aluminum phosphate, monocalcium phosphate, ignited natural aluminum phosphate, and superphosphate gave practically the same results. Likewise, the potassium metaphosphate, potassium pyrophosphate, and ignited synthetic aluminum phosphate produced almost as much growth as monocalcium phosphate. The calcium meta- and pyrophosphates, prepared by heating mono- and dicalcium phosphates, respectively, gave no increase in total plant growth. Attention has already been called to the behavior of the ferric phosphates and natural aluminum phosphate, the total yields of dry matter being in the same relative order as those obtained from the first cutting.

ABSORPTION OF P_2O_5 BY PLANTS

Portions of the dried and ground samples of plants from each cutting were fused with magnesium nitrate, and the phosphorus in the nitric acid extracts of the fusion residues was determined volumetrically by the molybdate method. The plants from the no-phosphorus jars contained 0.268, 0.247, 0.270, and 0.261 per cent P_2O_5 for the first, second, third, and total cuttings, respectively. In general, the plants fertilized with the various phosphates, except calcium metaphosphate, contained, at each cutting, higher percentages of P_2O_5 than those receiving no phos-

phate. The plants receiving calcium metaphosphate averaged slightly lower in P_2O_5 than those from the check jars.

The amounts of phosphorus actually taken up by the plants from the different phosphates are shown in Table 4. The results offer some interesting suggestions but cannot be compared directly with the amount of growth produced because external environmental factors may seriously affect the amount of growth produced from a given amount of fertilizer.

TABLE 4

Average net amounts of P_2O_5 absorbed by Sudan grass from various phosphates

SOURCE OF PHOSPHORUS	AVERAGE NET AMOUNT OF P_2O_5 ABSORBED			
	FIRST CUTTING	SECOND CUTTING	THIRD CUTTING	TOTAL
	mg.	*mg.*	*mg.*	*mg.*
Calcium Metaphosphate (heat)..............	8.2	1.1	−15.8	−6.5
Calcium Pyrophosphate (heat)......... ...	6.0	3.9	−2.5	7.4
Calcium Pyrophosphate (double decomposition)........	33.0	18.3	11.7	63.0
Potassium Metaphosphate (heat)....	46.5	28.9	15.6	91.0
Potassium Pyrophosphate (heat).....	51.7	27.0	15.8	94.5
Dimagnesium Phosphate...................	35.0	22.9	17.4	75.3
Trimagnesium Phosphate.....	48.1	24.3	16.9	89.3
Magnesium Ammonium Phosphate....	45.3	27.2	23.6	96.1
Ferric Phosphate.................	39.1	21.3	5.5	65.9
Ferric Phosphate, ignited...	26.1	11.5	−3.6	34.0
Aluminum Phosphate, synthetic....	60.2	22.2	2.1	84.5
Aluminum Phosphate, synthetic, ignited. ...	28.8	13.3	14.4	56.5
Aluminum Phosphate, natural..........	22.8	4.8	7.6	35.2
Aluminum Phosphate, natural, ignited.......	27.9	9.8	10.1	47.8
Monocalcium Phosphate............... .	37.1	18.8	11.2	67.1
Superphosphate....................	41.9	21.5	16.5	79.9
No phosphorus.....................	*	*	*	

* The plants from the check treatments contained 75.5, 68.7 and 57.2 mg. P_2O_5 at the first, second and third cuttings, respectively.

Therefore, the discussion of this part of the investigation will be limited entirely to the amount of phosphoric acid taken up by the plant, and its relation to the availability of the compound as shown by chemical analyses. In order to facilitate discussion, monocalcium phosphate will be taken as the standard. The results showing the negative and net small amounts of P_2O_5 taken up from the calcium meta- and pyrophosphates prepared by heating mono- and dicalcium phosphates, respectively, agree with the chemical test which shows a low percentage of citrate-soluble P_2O_5 in these compounds. On the other hand, the calcium pyrophosphate prepared by double decomposition had only 52 per cent of its P_2O_5 in a citrate-soluble form and yet the plants absorbed 94 per cent as much P_2O_5 as they did from monocalcium phosphate.

Plants absorbed more phosphorus from potassium metaphosphate, potassium pyrophosphate, trimagnesium phosphate, magnesium ammonium phosphate, unignited synthetic aluminum phosphate, dimagnesium phosphate and superphosphate than they did from monocalcium phosphate. The P_2O_5 in these compounds was all, or nearly all, citrate-soluble, and the absorption results agree with the chemical tests, in that both methods indicate a high availability for the P_2O_5.

Unignited ferric phosphate, which had all its P_2O_5 in a citrate-soluble form, gave up 98 per cent as much P_2O_5 as did monocalcium phosphate. Ignition of the ferric phosphate reduced the citrate solubility of the P_2O_5 to only 30.5 per cent, and the total amount of P_2O_5 absorbed by the plants from the ignited material was only 51 per cent of that absorbed from monocalcium phosphate.

The results from the aluminum phosphates are very interesting. Ignition of the synthetic aluminum phosphate did not affect the citrate solubility of the P_2O_5 but decreased the total amount of P_2O_5 absorbed by the plants. On the other hand, ignition of the natural aluminum phosphate increased the citrate solubility of the P_2O_5 and its absorption by the plants. The large absorption of phosphorus from unignited synthetic aluminum phosphate agrees with results reported by Brioux,[1] who found that aluminum phosphate was readily assimilated by flax, barley, spurry, buckwheat, mustard and yellow clover.

COMPARISON OF AVAILABILITY OF PHOSPHATES DETERMINED BY DIFFERENT METHODS

In Table 5 the relative fertilizer efficiencies of the phosphates, as shown by the increases in the total dry weights of the plants and the absorption of P_2O_5, are compared with their availabilities, as determined by the neutral ammonium citrate and citric acid methods. The increases in the total dry weights of the plants and the absorption of P_2O_5 are based on the increases obtained with monocalcium phosphate as 100.

With a few exceptions, the relative availabilities of the phosphates as determined by the citrate method are in good agreement with their relative fertilizer efficiencies as indicated by plant growth and absorption of P_2O_5, the absorption index usually giving values higher than those assigned by the other two methods. The results show, in general, that the citrate method gives a much better indication of the actual fertilizer value of iron and aluminum phosphates than is obtained by the citric acid method.

RELATIVE SOLUBILITY OF IRON AND ALUMINUM PHOSPHATES

A study of the amounts of P_2O_5 absorbed by the plants at each cutting (Table 4) suggests that there may be a difference in the rates of reactions

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[1] *Loc. cit.*

TABLE 5

Comparative availabilities and fertilizer efficiencies of various phosphates

MATERIAL	AVAILABILITY OF P_2O_5 AS DETERMINED BY—		FERTILIZER EFFICIENCY OF P_2O_5 AS INDICATED BY INCREASE IN—	
	NEUTRAL AMMONIUM CITRATE METHOD[a]	CITRIC ACID METHOD[a]	DRY WEIGHT OF PLANTS[b]	P_2O_5 ABSORBED BY PLANTS[b]
	per cent	*per cent*	*per cent*	*per cent*
Calcium Metaphosphate (heat)	0.0	0.9	−12.9	−9.5
Calcium Pyrophosphate (heat)	4.6	4.2	−30.0	11.1
Calcium Pyrophosphate (double decomposition)	52.2	79.2	40.0	94.0
Potassium Metaphosphate (heat)	97.6	57.6	81.5	135.7
Potassium Pyrophosphate (heat)	100.0	100.0	71.4	140.8
Dimagnesium Phosphate	100.0	100.0	63.5	121.2
Trimagnesium Phosphate	100.0	100.0	94.3	132.2
Magnesium Ammonium Phosphate	100.0	100.0	105.0	143.2
Ferric Phosphate	100.0	11.7	8.4	98.3
Ferric Phosphate, ignited	30.5	3.8	27.1	50.7
Aluminum Phosphate, synthetic	100.0	37.3	93.6	126.0
Aluminum Phosphate, synthetic, ignited	100.0	14.5	80.6	83.5
Aluminum Phosphate, natural	8.9	0.6	42.8	52.5
Aluminum Phosphate, natural, ignited	76.3	2.8	106.5	71.3
Monocalcium Phosphate	100.0	100.0	100.0	100.0
Superphosphate	98.5	92.1	85.8	119.0

[a] Based on the total P_2O_5 content of the material.
[b] Based on the increase from monocalcium phosphate as 100.

and solubilities of the compounds produced when iron and aluminum phosphates are added to soils. This is particularly true of the ignited compounds. The rate of solution measured by absorption of P_2O_5 by the plants is more uniform from the aluminum phosphates than from the ferric phosphates and more nearly approximates the results obtained from monocalcium phosphate. The results from the ignited synthetic aluminum phosphate show 28.8, 13.3, and 14.4 mg. of P_2O_5 taken by the crops at the first, second and third cuttings, respectively, whereas the crops absorbed 27.1, 18.8 and 17.2 mg. of P_2O_5 from monocalcium phosphate during the same periods, the results from ignited synthetic ferric phosphate being 26.1, 11.5 and −3.5 mg., respectively. This suggests that the compounds formed during the hydrolysis of aluminum phosphate are more soluble than those formed from ferric phosphates. These results tend to corroborate the findings of Austin[1] who reported that soluble phosphates added to soils may remain available to plants even though they are precipitated as aluminum phosphates.

[1] *Soil Science*, 24, 263 (1927).

Experiments were carried out to determine the solubility in water of the P_2O_5 of certain natural basic phosphates of iron and aluminum. The phosphates were museum specimens furnished by the Department of Geology, University of Arkansas. Inasmuch as the minerals were not available in sufficient quantities to make complete chemical analyses, it was assumed that their compositions corresponded to the formulas customarily assigned to them. It is evident, therefore, that these experiments must be considered as preliminary, and it is planned to carry out more extensive tests with minerals of known composition.

In making the tests the minerals were ground in an agate mortar and placed in a collodion bag in a weathered extraction flask, with redistilled water on both sides of the membrane. Samples of the diffusate were taken at the end of 24 hours, more water was added, and samples were taken again at the end of 72 hours. Phosphorus was determined colorimetrically in the dialyzed solution by the Denigès method.

TABLE 6

Solubility in water of certain iron and aluminum phosphates

MINERAL	COMPOSITION*	SOLUBLE P_2O_5	
		24 HOURS	72 HOURS
		p.p.m.	p.p.m.
Wavellite	$4AlPO_4 \cdot 2Al(OH)_3 \cdot 9H_2O$	0.062	0.041
Lazulite	$2AlPO_4 \cdot (Fe, Mg)(OH)_2$	0.051	0.062
Turquois	$AlPO_4 \cdot Al(OH)_3 \cdot H_2O + 2CuO \cdot P_2O_5 \cdot 4H_2O$	0.134	0.134
Dufrenite	$FePO_4 \cdot Fe(OH)_3$	Trace	0.045
Vivianite	$Fe_3(PO_4)_2 \cdot 8H_2O$	0.041	Trace

* Formulas given in Dana's "System of Mineralogy" 6th ed. (1914).

The results given in Table 6 indicate that the concentrations of water-soluble phosphorus liberated from the basic aluminum phosphates are high enough to support good growth of plants, if these concentrations can be maintained for a sufficient length of time. Tidmore[1] found that a concentration of 0.02 p.p.m. of the PO_4-ion in soil solutions produces good plant growth.

SUMMARY

The availability of various synthetic and natural phosphates, particularly the iron and aluminum phosphates, was determined by chemical methods, production of plant material and absorption of phosphorus by plants. The phosphates studied included synthetic and natural aluminum phosphates, ferric phosphate, di- and trimagnesium phosphates, magnesium ammonium phosphate, potassium and calcium pyrophosphates,

[1] *Soil Science*, 30, 13 (1930).

potassium and calcium metaphosphates, superphosphate and mono-calcium phosphate.

The magnesium phosphates, synthetic aluminum phosphate, unignited ferric phosphate, potassium meta- and pyrophosphates, and mono-calcium phosphate are completely or nearly completely soluble in neutral ammonium citrate solution according to the official method of analysis.

Ignition increases the citrate solubility of natural hydrated aluminum phosphate and decreases the solubility of synthetic ferric phosphate, but has no effect on the solubility of synthetic aluminum phosphate. The citrate solubility of ignited aluminum and ferric phosphates increases with increase in the weight of sample taken for analysis, whereas the reverse is true with unignited natural aluminum phosphate.

The relative efficiencies in production of increased growth of Sudan grass, as compared with monocalcium phosphate as 100, are for ignited natural aluminum phosphate 106.5, magnesium ammonium phosphate 105.0, trimagnesium phosphate 94.3, unignited synthetic aluminum phosphate 93.6, ignited synthetic aluminum phosphate 80.6, dimagnesium phosphate 63.5, unignited natural aluminum phosphate 42.8, ignited ferric phosphate 27.1 and unignited ferric phosphate 8.4.

The P_2O_5 of unignited synthetic aluminum phosphate, di- and tri-magnesium phosphates, magnesium ammonium phosphate, and potassium meta- and pyrophosphates is absorbed more readily by plants than is the P_2O_5 of monocalcium phosphate. For the other phosphates the relative increases in the amounts of P_2O_5 absorbed by the plants, compared to monocalcium phosphate as 100, are as follows: ferric phosphate 98.3, ignited synthetic aluminum phosphate 83.5, ignited natural aluminum phosphate 71.3, unignited natural aluminum phosphate 52.5 and ignited ferric phosphate 50.7.

In general, the relative availabilities of the phosphates as determined by the citrate method are in good agreement with their relative fertilizer efficiencies as indicated by plant growth and absorption of P_2O_5. On the basis of the total amount of dry matter produced, the only outstanding exceptions are the low value assigned to the unignited ferric phosphate which was completely soluble in citrate solution, and the high value assigned to the ignited natural aluminum phosphate. The results on the basis of amount of phosphorus absorbed by the plants also agree with the availability as shown by chemical analyses with the exception of the ignited synthetic aluminum phosphate and the unignited natural alumi-num phosphate. The results, as a whole, show that the neutral am-monium citrate test gives a reliable index of the fertilizer value of the P_2O_5 in these particular phosphates. The 2 per cent citric acid method does not give a reliable indication of the fertilizer value of the P_2O_5 in iron and aluminum phosphates.

DETERMINATIONS OF SMALL QUANTITIES OF FLUORINE

I. STEIGER-MERWIN REACTION—OPTIMUM CONDITIONS AND INTERFERENCE

By H. J. WICHMANN and DAN DAHLE (U. S. Food and Drug Administration, Washington, D. C.).

The use of compounds of fluorine as insecticides and the increasing knowledge of the danger to human health[1] involved in the daily consumption of minute quantities of fluorine in waters and food have created a demand for an accurate and specific micro method for its determination in waters, biological or toxicological material, and in spray residue on fruits and vegetables. As other investigators have responded to this demand with a number of published papers containing complete bibliographies, it is necessary here to refer only to those citations pertinent to this paper.

The quantity of fluorine found in potable waters varies from zero to 12–14 parts per million.[1] Sprayed fruits or vegetables prepared for market may contain similar quantities. There is, of course, a limit to the quantity of a food product that can be used for a fluorine determination. The Gutzeit[2] arsenic method specifies aliquot portions containing 0.01–0.03 mg. of As_2O_3. A satisfactory fluorine method should determine accurately similar quantities up to 0.1 mg., and to determine natural-occurring fluorine (as distinguished from added fluorine) in food products or fluorine in toxicological or biological materials the method should be accurate for even smaller quantities, 0.002–0.005 mg. for example. Therefore a colorimetric method appeared to be the only solution at the time these studies were begun.

Fluorine has a bleaching effect on many colored compounds, but unfortunately it is seldom specific. Usually a number of substances, both organic and inorganic, interfere by intensifying the color or by producing a similar bleaching effect. Therefore, the selection of the color reaction and consideration of possible interfering substances are of extreme importance.

Among the colored substances that are especially sensitive to fluorine, ferric thiocyanate and "peroxidized" titanium are outstanding. The influence of fluorine on peroxidized titanium has been studied by Steiger,[3] by Merwin,[4] and lately by Sharpless.[5] According to Fenton[6] di-hydroxy maleic acid develops a color 10–20 times more intense with titanium solutions than does hydrogen peroxide. However, this color is unstable and also extremely sensitive to other substances, for example, chlorides.

[1] H. V. Smith and Margaret Cammack Smith, Bulletins 32 and 43, University of Arizona.
[1] Methods of Analysis, A.O.A.C., 1930.
[3] J. Am. Chem. Soc., 30, 219 (1908).
[4] Am. J. Sci., (4) 28, 119 (1909).
[5] J. Nutrition, 6, No. 2, 163 (1933).
[6] J. Chem. Soc., 93, 1064 (1908).

The reaction of fluorine on ferric thiocyanate has been studied recently by Foster,[1] but when the writers began this investigation little was known about the effect of interfering substances. The peroxidized titanium reaction of Steiger, Merwin and Sharpless was selected for further study because these workers had already determined some of its possibilities and limitations.

OPTIMUM CONDITIONS

The Steiger-Merwin method was designed for the determination of fluorine in rocks. Aliquots containing 0.5–10 mg. of fluorine are used. This technic, however, is not sufficiently sensitive. Sharpless first isolated fluorine by volatilization as SiF_4, as advocated by Wagner and Ross,[2] and then determined the fluorine in aliquot portions of the distillate by a method more sensitive that the original Steiger-Merwin method. Early in the investigation it was noted that the yellow color produced by excess hydrogen peroxide on a definite quantity of titanium solution varied with the hydrogen-ion concentration. At a pH above 4 little, if any, color developed, but the shades of yellow darkened as the pH was lowered. Steiger, Merwin and Sharpless used strong sulfuric acid solutions, but they do not mention the influence of pH on the color value. The writers considered that with control of pH greater sensitivity might be obtained.

Determination of optimum pH with some degree of exactness requires a measurement of color that can be expressed quantitatively. A Nutting polarizing photometer graduated in terms of density

$$[D = \log. \, I_0/I, \, (I = \text{intensity})]$$

was available. Diffused light, obtained from a 400-watt lamp in a metal housing fitted with a ground-glass opening was passed (1) through two lenses to make the light rays parallel; (2) through a pair of 100 mm. glass tubes, one containing the solution to be examined and the other a "zero" solution (contained all the reagents except the titanium); (3) through the photometer; and (4) through a blue glass filter (Corning 53 Violet,) which showed maximum absorption. Ten closely agreeing settings of color density were made at a time, and the results were averaged. A peroxidized titanium solution similar to the one used by Sharpless was prepared and definite portions were treated with varying quantities of hydrochloric acid and then made up to a volume of 50 cc.

REAGENTS

(a) *Titanium solution.*—2 cc. of 20 per cent titanium chloride solution and 50 cc. of nitric acid (1+1) or hydrochloric acid (1+1) diluted to 1 liter with water.

(b) *Dilute hydrogen peroxide.*—5 cc. of 30 per cent hydrogen peroxide diluted to 100 cc. with water (2 cc. of diluted peroxide solution will provide hydrogen per-

[1] *Ind. Eng. Chem. Anal. Ed.*, **5**, 234 (1933).
[2] *Ind. Eng. Chem.*, **9**, 1116 (1917).

oxide in excess of the quantity required to bring out the maximum color in 1 cc. of the diluted titanium reagent.)

(c) *Hydrochloric acid.*—(1+9).

The color densities of the titanium solutions were measured against the color density of distilled water as zero. After the color density of the peroxidized titanium solutions had been obtained, the pH determinations were made colorimetrically with LaMotte standards and a block comparator. Densities were plotted against pH, and the curves A, B, and C (Fig. 1) were obtained. Curves A′, A″, B′, B″, and C′ are similar, but definite quantities of fluorine were present. The drop in color of the peroxidized titanium solutions in the pH range 1.25–2.50 is marked.

The bleaching action of fluorine increases with increasing pH to an optimum of approximately 1.5 and then rapidly decreases to practically zero at a pH of about 2.5. It is evident, therefore, that optimum pH is of great importance in the Steiger-Merwin method, and it would seem to be logical to believe that this would be true of other colorimetric methods that are based on the bleaching action of fluorine.

The next step was to determine the effect of volume and quantity of reagent (concentration) on the bleaching of fluorine at the optimum pH of 1.5. The data obtained are given in Figs. 2, 3, and 4. A study of these figures show (1) that the bleaching per unit of fluorine increases as the concentrations of fluorine increase and the concentrations of titanium decrease; (2) that up to certain limits it is proportional to the quantity of fluorine present; and (3) that above this limit the bleaching per unit of fluorine decreases. Concentration of both titanium and fluorine, therefore, has a strong influence on both the sensitivity and practical range of the reaction. The curves obtained by plotting percentage of bleaching vs. fluorine are similar to those obtained by Sharpless. Methods based on this reaction are flexible and can be adapted to varying conditions of sampling and analysis and to available apparatus. Curves E and F (Figs. 2, 3, and 4) show high sensitivity but limited range for fluorine (0–0.02 mg.). Curve A shows less than half the sensitivity but ten times the range. Under favorable circumstances (absence of interfering substances) this modified Steiger-Merwin reaction is as delicate as the Gutzeit method for arsenic.

INTERFERENCES

In a study of methods for the analysis of "things as they are" interferences occupy a prominent place. Steiger and Merwin listed phosphates, alkali sulfates, aluminum, and iron as interfering substances, and Merwin also declared that a change in temperature affects the results. If both standard and unknown solutions are compared at the same temperature, however, this factor is eliminated. Many inorganic and organic compounds besides those listed by Steiger and Merwin interfere

Fig. 1. Effect of pH.
A: 2cc "Ti" No F
A': " 0.05 mg F
A": " 0.15 mg F
B: 1cc "Ti" No F
B': " 0.05 mg F
B": " 0.10 mg F
C: ½cc "Ti" No F
C': " 0.05 mg F

Fig. 2. Effect of Volume and Quantity Ti-Sol.
A 2cc Ti-Sol. in 50cc Vol.
B 1 " 50 "
C ½ " 50 "
D ½ " 25 "
E 0.2 " 10 "
F 0.1 " 10 "

Fig. 3. Per cent Bleach as function of Volume and Quantity Ti-Sol.
A 2cc Ti " 50cc Vol.
B 1 " 50 "
C ½ " 50 "
D ½ " 25 "
E 0.2 " 10 "
F 0.1 " 10 "

Fig. 4
Read E and F Left
Read E' and F' Right

Fig. 5.
A: Std. Sol. + F
B: Std. Sol. + 10 mg P₂O₅/50 cc + F

Fig. 6.
A: Std. Sol. + no F
B: Std. Sol. + 0.015 mg. F
C: Std. Sol. + 0.045 mg. F
D: Std. Sol. + 0.075 mg. F
P₂O₅ variable

Fig. 7.

Effect of P₂O₅'s (H₃PO₄)
● Std.Sol.+F
× Std.Sol.+1.1mg.P₂O₅/50cc+F
▲ Std.Sol+275mg.P₂O₅/50cc.+F

Fig. 8.

● Std.Sol.+F
× Std.Sol.+0.05mg.Cl/50cc.+F
▲ Std.Sol+5mg.Cl/50cc.+F

Fig. 9.

A: Std. Sol.+ no F₂
B: Std. Sol.+0.04 mg.F
Al variable

Fig. 10.

A: Std.Sol.+F
B: Std.Sol.+0.5 g. K₂SO₄/50cc.+F

Fig. 11.

A: Std.Sol.+ no F
B: Std.Sol.+0.04 mg.F
K₂SO₄ variable.

Fig. 12.

A: Std.Sol.+F
B: Std.Sol.+2g.NaCl/50cc.+F

more or less. The coloring matter of fruits, even when oxidized to water white with hydrogen peroxide in alkaline solution is particularly objectionable in the determination of fluorine. Curves in Figs. 5–14 show in a quantitative way the interference of some compounds and the comparative non-interference of others. The interfering action of phosphorus pentoxide is shown in Figs 5, 6, and 7. One curve in Figs. 5 and 7 is the typical fluorine curve and the other shows the result of the simultaneous action of fluorine plus constant quantities of 1.1, 10.0 and 275 mg. of P_2O_5.

There is an unusual "crossing" of the curves in the neighborhood of 0.045 mg. of fluorine for the small quantities of phosphorus pentoxide and 0.025 for the large quantities. The presence of phosphorus pentoxide therefore causes a plus or minus error according to the quantity of fluorine. Fig. 6 shows the influence of varying quantities of phosphate on the color of peroxidized titanium in the absence and presence of fluorine. Curve C (Fig. 6) corresponding to the crossing point, is practically a straight line, that is, phosphates with this quantity of fluorine have practically no influence. In the presence of more, or less, fluorine, however, phosphates do have a considerable influence, but it is not constant. Interactions seem to occur with fluorine, titanium and phosphorus pentoxide and to depend upon their relative proportions. Hydrogen-ion concentration also has an influence upon these reactions as shown by the crossing point; at a *p*H of 1.0 the crossing shifts to the right (between 0.08 and 0.09 mg. of fluorine). Arsenates and borates also show the unusual "crossing," although to a lesser degree and the crossing is farther to the right.

The serious interference of aluminum is shown in Figs. 8 and 9. A minus error occurs in the fluorine determination when as little as 0.05 mg. of aluminum is present in 50 cc. This might be expected as aluminum and fluorine form one of the most stable of the numerous fluorine complexes. The curves in Fig. 9 illustrate the effect of varying quantities of aluminum on peroxidized titanium in the presence and absence of fluorine; 2 mg. of aluminum counteracts the bleaching effect of 0.04 mg. of fluorine. The fluorine apparently combines with the aluminum in preference to the titanium. There is, however, no crossing of the curves. Iron acts similarly to aluminum, but to a lesser degree. The color of iron salts naturally causes an interference of its own.

Steiger and Merwin state that sulfates partially restore the color of fluorine-bleached titanium solutions, that is, cause low results. Figs. 10 and 11 show curves obtained with potassium sulfate. Fig. 11 shows that sulfates have an influence on the color of peroxidized titanium and that 600 mg. of potassium sulfate in 50 cc. produces the greatest effect, as there are maxima in the curves at about that point. Curve B made with 0.04 mg. of fluorine is not exactly parallel to Curve A, made without fluorine. The curves in Figs. 10 and 11 indicate that sulfates cause only

minus errors but that there is some interaction with sulfates, titanium and fluorine so that their effects are not additive. Sulfates affect the Steiger-Merwin reaction much less than do phosphates.

The curves in Figs. 12 and 13 illustrate the slight interference of chlorides. Even in large quantities salt has little effect on the color of peroxidized titanium solutions. The small difference indicated by 2 grams of salt is checked by the two closely parallel curves of Fig. 13. Nitrate curves are similar to the chloride curves. Because chlorides or nitrates have much less influence on the reaction than do sulfates it is believed that a hydrochloric or nitric acid reagent is an improvement over the sulfuric acid reagent used by Steiger, Merwin, or Sharpless.

Fig 13
A: Std. Sol + 7loF
B: Std. Sol+0.04 mg F
NaCl variable

Fig. 14.
Effect of "Organic Materials."
A: Std. Sol. +F
B: Std. Sol.+ "Apple Wash" +F
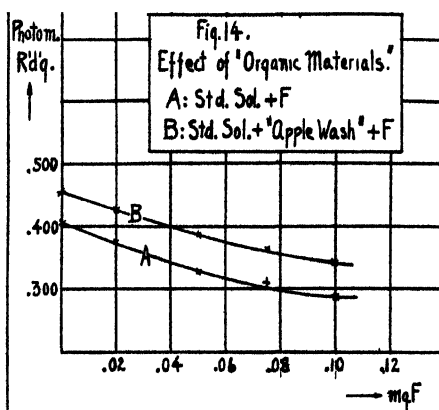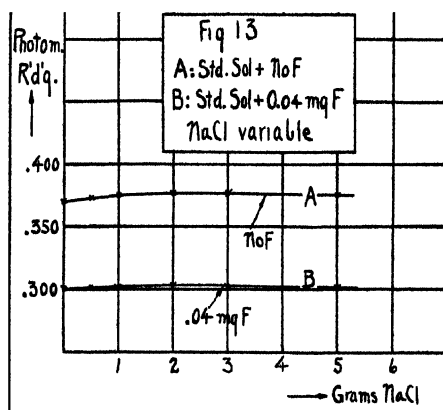
Calcium in the presence of fluorine has a slight effect on the reaction. The fluorine and the calcium-fluorine curves (100 mg. CaO) diverge slightly with increasing quantities of fluorine, the reaction no doubt being too acid for the formation of calcium fluoride. Small quantities of copper, such as might be found in spray residue, influence the curves but slightly when the color is measured by a photometer with a blue color filter, but when the colors are compared in Nessler tubes different shades are produced.

Fig. 14 illustrates the effect of organic matter derived from apple skins by the usual rapid methods of spray residual analysis. These involve treatment with hot dilute alkali and oxidation in alkaline solution with hydrogen peroxide. The oxidation destroys the coloring matter so that the solution is water white, but the simpler bodies formed, although practically colorless, have an appreciable effect on the titanium reagent. The "apple wash"-fluorine curve is parallel to the fluorine curve, but it is apparent that these organic derivatives will cause low results.

Many more curves could be made illustrating the positive or negative interference of other organic and inorganic compounds but there is no purpose in giving others. The interference of aluminum, iron, phosphates,

arsenates, sulfates and certain organic compounds is serious because these compounds are present in varying quantities in spray residues, waters, and biological material. Like quantities of interfering elements could be added to the standard solutions, but this would require additional complicated analyses. The same may be said about subtracting the effect of the interfering compounds upon the Steiger-Merwin reaction. This last expedient is especially dubious in view of the fact that the color density effects of fluorine and these interferences are not always additive. Parallel curves were found in only a limited number of instances; therefore it is believed that the best way to solve the question of interferences is first to isolate the fluorine (see following paper).

SUMMARY

(1) The Steiger-Merwin-Sharpless method was modified to include the optimum *p*H at which fluorine will bleach peroxidized titanium.

(2) The principles governing sensitivity and range of fluorine determination were definitely linked with concentration of titanium and fluorine, so that fluorine determinations might be sufficiently flexible to meet great variation in fluorine content and available apparatus. The principles stated should apply to other colorimetric bleaching methods for fluorine.

(3) The interference of such compounds as phosphates, aluminum, sulfates, and "apple wash" is quantitatively expressed.

(4) Some of these interferences suggest the formation of unknown complexes that prevent the accurate determination of fluorine because the effect of the foreign substance and fluorine on the titanium is not additive. What is true of the peroxidized titanium reaction is probably also true of other like reactions.

DETERMINATION OF SMALL QUANTITIES OF FLUORINE

II. STEIGER-MERWIN REACTION—DETAILS OF PROCEDURE

By H. J. Wichmann and Dan Dahle (U. S. Food and Drug Administration, Washington, D. C.)

In the preceding paper the writers discussed the theoretical factors controlling the optimum conditions of colorimetric fluorine determinations by the Steiger-Merwin-Sharpless peroxidized titanium method and the serious effect of certain interferences. They now present the details of a method of determination based on these factors.

Steiger[1] and Merwin[2] removed the interfering elements from rock by

[1] *J. Am. Chem. Soc.* **30**, 219 (1908).
[2] *Am. J. Sci.* No. 4, **28**, 119 (1909).

precipitation. Sharpless[1] first isolated the fluorine by volatilizing it as SiF₄ from ashed samples by a modification of the Reynolds-Ross-Jacobs[2] method, which requires the absence of water and therefore is slow and difficult. Willard and Winter[3] volatilized fluorine in the presence of water vapor by heating ashed samples to 135°C. with sulfuric or perchloric acid and determined the resulting H_2SiF_6 in the distillate by titration with thorium nitrate and zirconium-alizarine lake indicator.

The writers found the isolation procedure very satisfactory if 200 cc. is distilled, but preferred the colorimetric determination, especially for small quantities. However, the question of the superiority of either method under varying conditions is debatable and will be left for future consideration. It is also appreciated that the treatment accorded a food product or any other biological material prior to the Willard and Winter distillation is a problem in itself. For example, waters can be made alkaline and be concentrated in the distillation flask, but the preparation of fruits, vegetables or other organic materials for a fluorine determination is not such a simple procedure. Willard and Winter and Sharpless ashed their materials with a fluorine fixative prior to distillation, and Sharpless calls particular attention to the necessity of complete drying before ashing and to low ashing temperature. The success of the writers in isolating fluorine from fruits and vegetables without ashing by a double Willard and Winter distillation on (1) a subsample of the organic matter and (2) on the oxidized first distillate after evaporation leads to the opinion that the colorimetric method is adapted to either manner of preparation, but that the titrimetric determination requires preliminary ashing. The details of quick ashing in the presence of a catalyst[4] and of double distillation will also be discussed in later papers.

To determine fluorine colorimetrically the colors of the unknown solutions must be compared with the colors of solutions of known strength. Originally both Steiger and Merwin used Nessler tubes. The writers found Nessler tubes less satisfactory than a polarizing photometer, but the low cost of the tubes is often a determining factor. In the colorimetric method presented either a photometer or Nessler tubes may be used.

<div align="center">REAGENTS</div>

(a) *Standard fluoride solution.*—0.01 mg. of fluorine per cc. Prepare by diluting 10 cc. of a solution containing 0.100 mg. of fluorine as sodium fluoride per cc. to 100 cc. Use specially purified NaF or other pure soluble fluoride.

(b) *Hydrochloric acid.*—(1+9).

(c) *Titanium chloride solution.*—Dilute 2 cc. of 20 per cent TiCl₃ solution and 20 cc. of HCl to 1 liter.

(d) *Hydrogen peroxide solution.*—Dilute 5 cc. of 30 per cent H_2O_2 to 100 cc.

[1] *J. Nutrition,* 6 No. 2, 163 (1933).
[2] *This Journal,* 11, 225 (1928).
[3] *Ind. Eng. Chem. Anal. Ed.,* 5, 7 (1932).
[4] To be presented by R. U. Bonnar, San Francisco Station, U. S. Food and Drug Administration.

APPARATUS

(1) *Nessler tubes.*—50 cc. long form. Use at least 7, preferably more. (Tubes with fused bottoms of optical glass are desirable but not absolutely necessary.) Match ordinary tubes for length and test them for optical similarity by filling to the mark with a solution corresponding to the "0.04" standard described later. Reject tubes showing detectable differences in color or intensity.

(2) *Polarizing photometer.*—A photometer reading directly in terms of density is convenient but not necessary (see p. 613). Unless a spectral instrument is attached a color filter will be required (Corning 53 Violet). Two matched 100 mm. tubes to hold the unknown and "zero" solutions are necessary. If they hold slightly less than 10 cc. they can be used to read fluorine solutions varying from 10 to 100 cc., a decided advantage.

(3) *pH Comparator.*—Use any instrument that will show the pH of colored solutions to be equal to 1.50±0.02. (A special set made by the LaMotte Company has standards of 1.40, 1.45, 1.50, 1.55 and 1.60 pH units, with meta-cresol-purple indicator and a block comparator.)

(4) *Color standards.*—To each of six Nessler tubes add exactly 1.00 cc. of titanium solution (Reagent c) and approximately 1.30 cc. of hydrochloric acid. (Reagent b). The exact quantity of hydrochloric acid is that required to give a final pH of 1.50±0.02. It should be established for each lot of acid and be measured thereafter with great precision. Exact duplication of the quantity of titanium solution is also necessary owing to the influence of titanium concentration on the yellow color. Then add (1) 0.0, 1,0, 2.0, 3.0, 4.0 and 5.0 cc. of standard fluoride solution to six successive tubes, (2) 40 cc. of distilled water, and (3) 2 cc. of peroxide solution (Reagent d); make to the mark and mix by inverting the tubes. Test the pH of one of the series and replace. (If the tubes are kept stoppered they will keep constant for about 24 hours.) The quantity of the titanium solution may be increased or diminished according to the quantity of fluorine expected or the ability of the operator to match the colors. With less titanium and less total volume prepare standards to 0.03 mg. of fluorine and with larger quantities prepare to 0.10 mg. Sensitivity to fluorine varies inversely with the concentration of the titanium but the range varies directly.

Total volume and volume of reagents can be adjusted to suit other methods of comparing color if necessary. As diminishing the titanium increases the sensitivity, it follows that the pale yellow solutions must be viewed through as long a column as possible. Colorimeters of the Shreiner type require comparatively large volumes and their use would therefore be limited to relatively large quantities of fluorine in the aliquot portions taken. Long prism colorimeters of the Bausch and Lomb type might also be suitable under some circumstances. Sharpless used a micro-colorimeter of the short plunger type. To obtain sufficient color and at the same time sensitivity, he kept his volumes very small (5 cc.). If colorimeters are preferred, a micro model, with cups holding 10 cc. or less, and long plungers (100 mm.) would perhaps be ideal. When lower-priced photo-electric instruments that give reproducible and accurate results are available they should be investigated.

DETERMINATION

Mix the Willard and Winter distillate, filtering if necessary, and place in a Nessler tube an aliquot portion calculated to contain between 0.01 and 0.05 mg. of

fluorine. The foregoing procedure is designed for the usual amounts of fluorine present as spray residue. When the fluorine content is markedly smaller or greater, the quantities of reagents must be varied to correspond to the appropriate curve in Figs. 2, 3 and 4. The analyst should determine whether range or sensitivity is the most important consideration, and vary the concentration of fluorine and of titanium accordingly. If the fluorine content is exceedingly small, it may be necessary to concentrate the distillate, after it has been made distinctly alkaline with sodium hydroxide, to a point where Curve B, or a curve of greater sensitivity, is applicable. Add 1.00 cc. of the titanium solution and 1.30 cc. of the hydrochloric acid and fill nearly to the mark with water. Then add 2 cc. of the peroxide solution, fill to the 50 cc. mark, and mix by inverting the tube. Determine the pH.

Samples containing appreciable percentages of chlorides or nitrates (naturally, or added) will introduce mineral acid into the Willard and Winter distillates and affect the volume of hydrochloric acid necessary for proper adjustment of pH.

In this case the volume of adjusting acid must be determined by experiment. Volatile organic acids have little influence on the pH. After the correct quantity of acid to give a pH of 1.50 ± 0.02 in the final solutions has been ascertained, repeat the preparation of a sample tube, making such adjustments of the volume of the hydrochloric acid as may be necessary, and compare with the standards. Compare the sample tube with two adjacent standard tubes until a match is obtained, interpolating if necessary. It is convenient to use a three-compartment box comparator, placing the sample tube between the two standard tubes. Such a comparator is easily constructed. Uniform moderate illumination, a minimum of color inter-

TABLE 1

Fluorine recovery

SAMPLE	ADDED	RECOVERED
	mg.	*mg.*
Apple Peelings	None	None
Apple Peelings	0.50	0.47
Apple Peelings	0.50	0.50
Apple Peelings	0.30	0.31
Apple Peelings	0.25	0.25
Apple Peelings	0.478	0.484
Apple Peelings	0.40	0.40
Apple Peelings	0.40	0.39
Cabbage	None	None
	0.249	0.248
	per cent	*per cent*
Rat Feed*	0.01 NaF	0.010 NaF
	0.02 NaF	0.018 NaF
	0.03 NaF	0.032 NaF
	0.05 NaF	0.050 NaF
	0.10 NaF	0.118 NaF
	0.01 Na_2SiF_6	0.012 Na_2SiF_6
	0.05 Na_2SiF_6	0.050 Na_2SiF_6
	0.0006 NaF	0.00066 Na_2SiF_6
	0.0012 NaF	0.0010 NaF
	0.0025 NaF	0.0022 NaF
	0.005 NaF	0.0048 NaF

* Prepared by Floyd De Eds, U. S. Bureau of Chemistry and Soils, San Francisco, California.

ference by shadows or surrounding objects, and a minimum of eye fatigue are necessary for satisfactory results. If the color of the unknown does not fall between the 0.01 and 0.05 standards (straight line portion of curve B (Fig. 2), it will be necessary to use another aliquot portion and a different quantity of the acid for adjustment of pH, and it is always necessary to check the pH before the final calculation of results. It should be 1.50 ± 0.02.

TABLE 2

Fluorine content of various foods determined by the colorimetric method

SAMPLE	FOUND	GRAIN PER LB.
	p.p.m.	
Drinking water (Arizona)	2.2	
Drinking water (Arizona)	3.92	
Drinking water (Arizona)	1.36	
Drinking water (Arizona)	0.45	
Ca-monophosphate	307.	
Ca-diphosphate	281	
Ca-triphosphate	256	
Apples, sprayed, unwiped	2.0	0.014
Apples, sprayed, wiped	1.0	0.007
Cabbage sprayed:		
No. 1. 7 outside leaves,	33.6	0.235
remaining head	2.7	0.019
No. 2. entire head	1.7	0.012
Broccoli (probably sprayed)	0.3	0.002
Tomatoes, sprayed	2.0	0.014
Tomatoes, sprayed	3.9	0.027
Celery sprayed		
No. 1: Petioles	7.6	0.053
Leaves	77.1	0.540
No. 2: Petioles	3.6	0.025
Leaves	135.3	0.947
No. 3: Petioles	1.7	0.012
	5.6	0.039
Strawberry juice (F used as preservative)	141.0	

Practically the same procedure is required if the color comparisons are made with a photometer except that fewer standards are required. Curves similar to those in Figs. 2, 3, or 4, p. 615 can be prepared and the fluorine read from them. Sufficient standards should be read to assure that the "set" of the instrument, remains unchanged. Necessarily the temperature should be the same when the unknown sample is read as when the curve is made. The writers read two standards corresponding to 0.00 and 0.05 mg. of fluorine whenever these determinations are made, interpolating the unknown mathematically. This is permissible because 0.00 and 0.05 mg. of fluorine define the straight line portion of the fluorine curve where 1 cc. of titanium solution is used in 50 cc. volume. Any necessary changes will readily suggest themselves to the analyst if the other optical instruments are used for comparison.

RESULTS

Data on the determination of fluorine in a number of foods are given in Tables 1 and 2; those in Table 1 represent recovery experiments and those in Table 2 show some of the quantities of fluorine recovered from a small but selected number of foods.

Fluorine occurred in the samples examined in four different ways: (1) In drinking water as a natural ingredient; (2) in the manufactured phosphates as a remnant of that in the original phosphate rock; (3) on the apples and vegetables as spray residues; (4) in the strawberry juice as a preservative.

SUMMARY

1. The details of a method for the determination colorimetrically of small quantities of isolated fluorine are presented. The method has wide application.

2. Typical results are given.

CHEMICAL STUDY OF TOMATO JUICE

By CHARLES F. POE, ARTHUR P. WYSS, and TRUMAN G. MCEVER
(State Food and Drug Laboratory and Department of Chemistry, University of Colorado, Boulder, Colorado)

The manufacture of canned tomato juice, which was begun on a commercial scale in the fall of 1929, has increased so rapidly that this beverage has become an important food product.

The Food and Drug Administration, U. S. Department of Agriculture, states that tomato juice is made by two processes. One process consists in putting the tomato through a fine sieve, which leaves only the skin, core, and seeds as residue, and the other in using only the soft part of the tomato. Usually, from 0.25 to 1.0 per cent of salt is added. Some manufacturers also add from 1 to 2 per cent of sugar, which helps to increase the viscosity of the product and to maintain a more even distribution of pulp and liquid. Steenback and Schrader[1] show that the whole juice is approximately thirty-two times richer in vitamin A than the filtered juice. For this reason it would appear that the juice should contain all the pulp, thereby representing so far as possible the whole tomato.

In view of the varying methods of manufacturing, the high vitamin content of the pulp, and the lack of standards whereby the analyst may judge this product, it was thought important to investigate a number of tomato juices of home production.

The tomatoes were obtained from different sources in Colorado during the season of 1931. Part of them were carefully washed, quartered,

[1] *J. Nutrition*, 4, 267 (1931).

crushed, quickly brought to the boiling point in a large covered kettle, and run through a rotary sieve which removed practically all the pulp and liquid. Adhering water was removed after the washing process, and all blemishes and spots were cut out. The product was then put through a 20-mesh sieve, placed in quart jars, and sterilized in live steam for 30 minutes. With two lots, one-half of each tomato was made into juice by the above process, and the other half was used to prepare a raw juice. A comparison between the raw and cooked juices was thus made possible.

The methods of analysis used were essentially those given in *Methods of Analysis*, A.O.A.C. Total solids were determined on the original and on the filtered juice (serum). In each case the sample was mixed thoroughly. The percentage of insoluble solids was obtained by direct determination as well as by difference. All determinations except the total

TABLE 1
Composition of juices from raw and cooked tomatoes
(Results expressed in percentage)

CONSTITUENTS	RAW	COOKED	RAW	COOKED	RAW	COOKED
Total solids	5.01	5.12	5.60	5.62	5.59	5.64
Soluble solids	4.56	4.70	5.18	5.16	5.14	5.18
Insoluble solids	0.47	0.44	0.44	0.49	0.47	0.49
Ash	0.48	0.48	0.51	0.51	0.39	0.38
Water-soluble ash	0.43	0.40	0.46	0.45	0.36	0.34
Insoluble ash	0.05	0.08	0.05	0.06	0.03	0.04
Alkalinity of water-soluble ash*	4.82	4.97	4.05	3.95	5.34	5.28
Salt	0.06	0.06	0.08	0.06	0.02	0.02
Index of refraction n_D 20° C.	1.3401	1.3400	1.3411	1.3409	1.3408	1.3407
Immersion refractometer reading 20° C.	32.8	33.0	35.7	35.1	34.9	34.6
Specific gravity 20° C.	1.0204	1.0200	0.0224	1.0220	1.0223	1.0227
Acetic acid†	0.78	0.80	0.76	0.79	0.82	0.85
Fixed acid†	68.5	67.0	60.4	58.0	60.0	61.1
Fixed acid as malic	0.46	0.45	0.40	0.39	0.40	0.41
Reducing sugar	2.24	2.29	3.25	3.20	3.26	3.26
Reducing sugar after inversion	2.31	2.36	3.30	3.28	3.32	3.33

* cc. N/1 HCl per 100 grams of juice.
† cc. N/10 NaOH per 100 grams of juice.

solids and insoluble solids were made on the filtered juice (serum). For the determination of sugar, the Munson and Walker gravimetric method was used with the first samples. Later the Folin-Wu[1] and the dinitrophenol[2] methods were also used as checks. The results recorded in the tables were obtained by the Munson and Walker method.

Table 1 gives the results for the raw and cooked juices from three

[1] *J. Biol. Chem.*, **82**, 38 (1929).
[2] Poe and Edson. *J. Ind. Eng. Chem. Anal. Ed.*, **4**, 300 (1932).

varieties of tomatoes. Practically no difference can be noted in the constituents found in the raw and cooked juice made from the same sample.

Sixteen additional samples of home-canned juices were next analyzed. The results for the samples with no added salt are given in Table 2, and those for the samples containing added salt are listed in Table 3.

TABLE 2

Composition of home-canned tomato juices

(Results expressed in percentage)

CONSTITUENTS	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	NO. 7	NO. 8	AVER-AGE
Total solids	5.51	5.41	5.14	5.02	6.02	6.02	6.07	6.48	5.71
Soluble solids	5.09	5.02	4.76	4.66	5.72	5.50	5.61	6.10	5.31
Insoluble solids	0.44	0.41	0.40	0.38	0.32	0.55	0.49	0.40	0.43
Alcohol soluble solids*	64.00	63.80	58.77	66.12	55.55	54.93	58.60	53.98	59.47
Ash	0.45	0.41	0.49	0.51	0.45	0.46	0.49	0.48	0.47
Water-soluble ash	0.42	0.39	0.46	0.48	0.42	0.43	0.47	0.43	0.44
Insoluble ash	0.03	0.02	0.03	0.05	0.03	0.03	0.02	0.05	0.03
Alkalinity of water-soluble ash†	5.87	6.26	5.15	4.65	6.34	6.53	6.28	6.11	5.90
Salt	0.06	0.08	0.10	0.12	0.11	0.09	0.10	0.07	0.09
Index of refraction n_D 20°C.	1.3406	1.3408	1.3406	1.3400	1.3417	1.3416	1.3418	1.3424	1.3412
Immersion refractometer reading 20°C.	34.5	34.7	34.2	32.8	37.2	37.0	37.5	39.1	35.9
Specific gravity 20°C.	1.0223	1.0218	1.0209	1.0203	1.0248	1.0239	1.0248	1.0267	1.0232
Acetic acid‡	0.77	0.67	0.72	0.68	0.77	0.72	0.61	0.76	0.71
Fixed acid‡	58.6	57.7	74.5	44.1	79.1	67.4	66.9	62.1	63.8
Fixed acid as malic	0.39	0.39	0.50	0.30	0.53	0.45	0.45	0.42	0.43
Reducing sugar after inversion	3.04	3.08	2.44	3.04	3.38	3.12	3.26	3.25	3.08

* Reported as the percentage of total solids (serum) soluble in 95% alcohol.
† cc. $N/1$ HCl per 100 grams of juice.
‡ cc. N/10 NaOH per 100 grams of juice.

TABLE 3

Composition of home-canned tomato juices containing added salt

(Results expressed in percentage)

CONSTITUENTS	NO. 1	NO. 2	NO. 3	NO. 4	NO. 5	NO. 6	NO. 7	NO. 8	AVER-AGE
Total solids	7.90	7.85	7.37	6.37	7.41	6.43	6.96	7.61	7.24
Total solids salt-free	6.42	6.31	5.32	5.75	6.37	5.67	5.22	6.18	5.91
Soluble solids	7.44	7.34	6.92	6.04	6.98	5.99	6.65	7.18	6.82
Soluble solids, salt-free	5.96	5.80	4.87	5.42	5.94	5.23	4.91	5.75	5.49
Insoluble solids	0.50	0.55	0.49	0.35	0.46	0.47	0.33	0.46	0.45
Alcohol-soluble solids*	57.25	57.00	55.62	62.60	54.93	55.55	59.12	64.12	58.27
Salt-free, ash	0.42	0.41	0.50	0.42	0.43	0.34	0.42	0.43	0.42
Salt	1.48	1.54	2.05	0.62	1.04	0.76	1.74	1.43	1.33
Index of refraction n_D 20°C.	1.3447	13.448	1.3440	1.3424	1.3436	1.3421	1.3433	1.3442	1.3436
Index of refraction n_D 20°C. (corrected for salt)	1.3420	1.3421	1.3407	1.3413	1.3418	1.3407	1.3403	1.3418	1.3414
Immersion refractometer reading 20°C.	45.2	45.3	43.7	39.0	42.3	38.4	41.4	43.6	42.4
Immersion refractometer reading 20°C. (corrected for salt)	38.3	38.4	34.5	36.2	37.6	34.8	33.2	36.9	36.3
Specific gravity 20°C.	1.0361	1.0356	1.0354	1.0286	1.0319	1.0278	1.0327	1.0346	1.0328
Specific gravity 20°C. (corrected for salt)	1.0255	1.0250	1.0212	1.0243	1.0248	1.0224	1.0204	1.0244	1.0235
Acetic acid†	0.58	0.80	0.70	0.83	0.69	0.80	0.78	0.82	0.75
Fixed acid†	70.2	72.2	68.6	55.0	81.2	69.6	75.1	62.1	69.3
Fixed acid as malic	0.47	0.48	0.46	0.37	0.54	0.47	0.50	0.42	0.46
Reducing sugar after inversion	3.02	2.96	2.46	3.15	3.28	3.05	2.26	3.01	2.90

* Reported as the percentage of total solids (serum) soluble in 95% alcohol.
† cc. $0.1N$ NaOH per 100 grams of juice.

The results for insoluble solids on the whole juice and of total solids on the serum are consistent. The serum solids ran from 4.56 to 6.10 per cent and the insoluble solids from 0.32 to 0.55 per cent. The values for total

solids compare favorably with results given by Bigelow,[1] and by Saywell and Cruess[2] who studied the composition of various kinds of tomatoes. The values for ash, water-soluble solids, alkalinity of ash, and acetic acid vary within a very narrow range. The results for the immersion refractometer reading, malic acid, and reducing sugars indicate a lesser constant composition.

A study of all the tables indicates that a number of characteristic determinations might be used as a guide for the detection of adulteration: namely, total solids and insoluble solids on the whole juice; solids, solids soluble in water, ash, alkalinity of ash, immersion refractometer reading, volatile acids, and reducing sugars on the serum.

Any results reported in this communication that may be used as standards for judging commercial samples should be considered as tentative. A large number of samples from widely different sources and from several seasons should be studied before satisfactory conclusions can be drawn.

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[1] *This Journal*, **3**, 1 (1917).
[2] Univ. California Agr. Exp. Station Bull, 545 (1932).

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